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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

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THE OCCASION OF THE TWENTY FIRST EXTRAORDINARY CONGRESS OF THE COMMUNIST PARTY

On January 27, 1959 is to be summoned the 21st extraordinary congress of the Communist Party of the Soviet Union in order to discuss the control figures for the development of the national economy of our country in the years 1959-1965.

In the time which has elapsed since the 20th congress of the Communist Party, socialist economics, science and culture have achieved new successes in our country, which has made possible the further growth of the material welfare of the population. The resolutions passed at the 20th congress of the Communist Party, and the carrying out of these resolutions have laid the way for these successes, of which many examples are to be seen. At the present time the Soviet Union has surpassed all the capitalist countries of Europe in the level of its industrial production, and in the tempo of its growth it has outstripped such a highly developed industrial country as the United States of America.

Only last year the USSR was very close to the USA in its output of a number of agricultural products, and in a few of these it has already caught up to or even surpassed the present level of production of these products in the USA. The present year, 1958, has witnessed the achievement of some particularly important result in the field of agriculture in our country; the harvest of grain and of several other important crops has been on an unprecedented scale. Because of the achievements of our country in the realms of industry and agriculture, the national income of the USSR has increased.

The exploits of Soviet scientists, and of all our people, have aroused the universal admiration and acknowledgement of all progressive mankind. In August, 1957 was launched the first long range intercontinental rocket capable of reaching any part of the earth's surface. On October 4, 1947, which has become a historic data in the life of progressive mankind, the first artificial earth satellite was launched. One month afterwards an even more audacious plan was carried out – the launching into the cosmos of an entire scientific laboratory, on board which was the first living creature to ascend into the cosmos. On 15 May, 1958 the third Soviet artificial satellite reached an even greater height. The data received as a result of the launching of three artificial earth satellites is of exceptional value to the science of the whole world. The Soviet Union has been highly successful in the practical use of atomic energy for peaceful purposes. The first electric power station in the world worked by atomic energy was put into action in the Soviet Union. The first atomic icebreaker was constructed in the Soviet Union, and in April 1957 the most powerful synchrophasotron – an accelerator of nuclear particles – in the world began its work in the Soviet Union. Finally the first powerful jet aircraft to be used on passenger lines in the world were introduced in the Soviet Union. The discoveries of our scientists in the Arctic and Antarctic are widely known. Scientists from our country are the first speakers at the international tribunal on controlled thermonuclear reactions.

In the Soviet Union nuclear radiation is widely used for its action on various processes taking place in the body. Radioactive isotopes have found wide application in our country in industry, agriculture, biology and medicine. Soviet science is making a valuable contribution to the technical progress of our country, and to its economic and cultural development, and to the strengthening of the defensive forces of the socialist state. Its outstanding successes in the fields of mathematics, physics, chemistry, electronics, etc. have led to the solution of the most complicated problems in the development of power engineering, machine construction, metallurgy, radiotechnics, automation and telemechanics, and to the introduction into industry of the latest technics and to the development of the most highly efficient technological processes.

The grandiose plans for the development of the economy, culture and science of our country outlined in the

speeches of comrade N. S. Khrushchev to the 21st extraordinary congress of the Communist Party of the Soviet Union give evidence of the new and gigantic race of our country towards communism. These plans inspire the entire Soviet people and all Soviet scientists to further venture and to successful struggles in the building up of communism. In this new 7-year plan for the future great attention has been paid to the development of medicine and biology.

The achievements of our country in the development of a socialist economy have facilitated further progress in the field of the technical sciences, physics and mathematics and have created favorable conditions for the successful development of biology and medicine.

During the last 10-15 years, Soviet doctors and biologists have enriched our medical science with many valuable pieces of research, which have laid the foundation for new successes by the public health departments in the further improvement of the health of the population of our country, the further reduction in the general mortality and the systematic reduction in the morbidity.

In their activities Soviet medical scientists are guided by the noble purpose of standing guard over the health of the working population, to prevent disease and not only to treat it, and to carry out extensive health measures. The development of Soviet science, and in particular of branches such as biology, physiology and pathology, have made it possible to penetrate deeply into the essence of vital phenomena.

The development of Soviet medicine has taken place on the foundation of the Soviet theory of the influence of the nervous system. To take into account the special role of the nervous system as a system unifying all the functions of the body enables an understanding of the mechanism of the most diverse processes.

Soviet physiologists have continued to utilize and develop the ideas of I. P. Pavlov and have greatly increased our knowledge by their studies of the mechanisms of the higher nervous activity of man and animals. By making good use of modern methods of electrographic analysis of the processes taking place in the central nervous system, physiology in our country has applied the results which have accumulated to the treatment of a number of diseases of the central nervous system. Modern neurosurgery, neuropathology and psychiatry make extensive use in diagnosis and treatment of several facts established by physiologists. Other sections of physiology have yielded equally fruitful results. The joint working of physiologists and clinicians has developed successfully. Nowadays it is impossible to imagine any major hospital or clinic in which physiological methods of examination of the function of the circulation, respiration, digestion and so on are not practised. It has become the custom for out clinical hospitals to have physiological laboratories to assist in the diagnosis and treatment of disease.

Considerable success has been attained by Soviet pathologists in the study of the mechanisms of disease and recovery, and in the theoretical analysis of diseases such as hypertension, peptic ulcer and some infectious diseases. In this way a characteristic feature of the development of these two disciplines has been the significant penetration into pathology of physiological methods of analysis of pathological processes. In their turn, physiologists have also studied and investigated physiological processes by making use of the wide range of methods of pathology.

A conspicuous place in research has been occupied by work on ontogenesis and age characteristics of higher nervous activity. This research has resulted in the collection of some very valuable material on the subject of the age characteristics of physiological and pathological processes.

In the 7-year plan for the future development of Soviet medical science, new tasks have been placed before physiologists and pathologists, the solution of which should lead to a significant advance in theoretical and practical medicine. A special feature of the research which is planned here is not only the mutual penetration of physiology and pathology, but the maximum association of these subjects to the requirements of public health. One of the most important conditions for the successful solution of the tasks which are laid down is the continual raising of the standard of the methods and technical equipment of experimental research.

Further advances have been made in the field of pharmacology. The more profound study of the nervous regulation of the functions and reactivity of the individual links of the reflex arc in relation to various chemical substances has enabled the synthesis or isolation from medical plants of a whole series of new drugs (spasmolytics, curare-like drugs, cardiac drugs and many others) possessing the property of action on individual mechanisms of effection and regulation of physiological functions in normal and pathological conditions.

New antibiotics and synthetic chemotherapeutic preparations have been obtained and studied. Progress has

been made in the field of obtaining drugs for vitamin and hormone therapy. The plan for the future calls for the further development of this work in order to completely equip Soviet medicine with effective drugs of low toxicity for the treatment of the most diverse infectious and noninfectious diseases.

Very recently real progress has been made in the study of the problem of premature senility, and the special features of the physiology and pathology of old age. These tasks will be undertaken particularly by the newly organized Institute of Gerontology of the AMN SSSR.

The study of the structure and function of protein and its role in metabolism plays a prominent part in the development of medical biology in our country. The progress of research in this direction has led to the accumulation of some very valuable data on the composition and structure of proteins, on individual proteins, on ways of conversion and synthesis of proteins in the body, and the role of proteins and their functions in the tissues of the nervous system. The results obtained indicate new lines for research in this field.

Soviet microbiologists have done a great deal of research on the laws of variation of microorganisms and have studied its importance in biology and medicine. The facts which have been gathered provide the basis for proper notions of the evolution of living creatures and shows the way to develop this problem so that it may be put to practical use and also solve some fundamentally important theoretical problems. A characteristic feature of the development of this particular problem during the last five years was the determination of the possibilities of artificial interference in the processes of formation of species of bacteria, yeasts and molds of practical importance, with the aim of modifying their hereditary nature. Much of the experimental work on Soviet microbiologists was directed towards the establishment of the principle in accordance with which the external environment, under certain conditions, creates new properties and external signs in an organism and does not merely bring to light existing features as determined by heredity.

In recent years new experimental and clinical findings have been gathered on the nature and role of the so-called precancerous diseases in the origin of malignant tumors. The study of this problem has led to an approach being made to the problem of the prophylaxis of malignant tumors in man. Research on the immunology of cancer, the detection and removal of carcinogenic factors in the external environment have led to the separation of cancer prophylaxis as an independent problem. The development of a method of chemotherapy and hormone therapy of tumors is of great importance. Research in this direction must occupy a prominent position in the plan of future work. Since the problem of cancer itself is not purely medical but biological as well, its solution must concern institutions of various types, and increasingly wide participation in this research must be given by representatives from biology, biochemistry, chemistry, physics and other specialties.

Many changes have taken place in recent years in the field of study of regeneration, which is of great importance in medicine and, especially, in surgery. Whereas formerly reparative regeneration was studied mainly in cold-blooded animals, nowadays the main test objects have become mammals, which brings this division of biology closer to medicine. In the plan for future research on this problem great attention must be paid to physiological regeneration, whose importance in normal and pathological physiology has long been underestimated. This problem must be treated not so much from a general biological as from a medical aspect.

Discussions in the field of genetics have aroused great interest in inherited changes in somatic cells, which have made it possible to make use of genetic data in studying the mechanisms of malignant growth and have shed light on certain problems of homografting of tissues. At the same time there has been a considerable lagging behind in the study of the role of heredity in the development of certain diseases. The widespread use of atomic energy has stimulated the development of radiation genetics and has posed a number of new tasks before Soviet biologists and doctors.

Research in the field of embryonic development of living creatures is sharply subdivided at the present time. Thus, we have the physiology and biochemistry of embryonic development, the immunology of embryogenesis, the dynamics of embryonic development and so on. The most interesting for medicine at the present time are the biochemistry and immunology of embryogenesis, since purely immunological forms of pathology of intra-uterine development of man have been discovered, and since the immunological and biochemical features of embryogenesis which have attracted the attention of all biologists may lead to an explanation of some of the peculiarities of development of cells and tissues in embryogenesis.

These lines of activity in medical biology are extremely topical, and must be the subjects of the most concentrated attention in the coming years.

The research carried out by Soviet biologists and medical scientists in the fields of development both of medical theory and practice have borne real fruit. These have been reflected in the statistics of the birth rate and the expectation of life, and in the general physical development of the population. The birth rate and the natural increase of the population are higher in our country than in the leading capitalist countries.

The general mortality rate of the population of the USSR has fallen to one quarter of its prerevolutionary level, and the infant mortality rate has fallen 6.1 times. In the years of the fifth five-year period alone the population of our country has increased by 16.3 million persons, which is equal in number to the combined population of Sweden, Norway and Finland. The mean longevity of the population of the USSR, from the figures for 1955-1956, had more than doubled the figures for the prerevolutionary period. Morbidity and mortality have sharply declined.

The eradication of a number of diseases, particularly infectious, has been placed on a scientific footing. Nowadays students of Soviet medical institutes learn about certain diseases only from books, being unable, happily, to study them in practice.

The forthcoming extraordinary 21st congress of the Communist Party of the Soviet Union will discuss the control figures for the development of the national economy of the USSR in 1959-1965. In these figures will be reflected the results of the building of socialism in our country, and in particular the planned future progress of the country on the path towards communism. The congress will work out a militant program for a new and powerful upsurge in the socialist economy, culture and welfare of the people.

The new plan for the future development of the national economy of our country, which will be the subject for discussion at the 21st congress of the Communist Party, will undoubtedly play a tremendous part in the development of Soviet medicine and biology.

In its program, the Communist Party of the Soviet Union makes great demands on medical science, its theory and practice. The main task which is put before Soviet medicine is to stand guard over the health of the working population, and not only to treat but to prevent disease.

With this summary of certain of the positive achievements of our work, Soviet doctors and biologists must not forget that ahead of them lie many more unsolved problems. However, there is no doubt that the rich experience of the development of Soviet medicine will open up new and even greater prospects of the successful campaign for the health of the Soviet citizen and for the universal development of all his physical and spiritual powers.

PHYSIOLOGY

REFLEX CHANGES IN THE ACTIVITY OF THE CARDIOVASCULAR SYSTEM DUE TO CHEMICAL STIMULATION OF THE PERICARDIAL RECEPTORS

COMMUNICATION III. THE DEPENDENCE OF THESE CHANGES ON THE STATE OF THE VASOMOTOR CENTER

B. S. Kulaev

From the Laboratory of General Physiology (Head – Active Member of the AMN SSSR V. N. Chernigovskii) of the Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) of the AMN SSSR, Moscow

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In a survey of the finding on the nervous regulation of the tone of the blood vessels, Folkow[11]comes to the conclusion that an increase in the activity of the vasomotor center leads to an increase in the efferent impulses to the vessesl, as a result of which the tone of the vessels rises; inhibition of the vasomotor center is expressed as weakening of the efferent impulses, dilatation of the vessels and a fall in the blood pressure. Other things being equal, the state of a nervous center is a function of the number of afferent impulses reaching it. We have shown that chemical stimulation of the receptors of the pericardium of cats in the majority of cases causes in the same experiment both pressor and depressor reactions of the cardiovascular system, and that the former appear in response to stimuli of relatively low intensity whereas the latter are in response to more intensive stimuli; however, in some experiments pressor or depressor reactions only were observed (see Communication I). Exclusion of all the receptors or of the chemical receptors only in the pericardium always causes a rise in the blood pressure [7, 8].

Our findings compel us to disagree with the notion of the qualitative heterogeneity of afferent influences causing excitation or inhibition of the vasomotor center. These facts may explain, if they are accepted, how a different number of impulses could cause qualitatively opposite states at the place of their application [1, 5, 6].

For this explanation to be acceptable it is essential in the first place to prove that the character of the reaction of the cardiovascular system to stimulation of the receptors of the pericardium depends on the state of the vasomotor center and, secondly, that a change in the number of afferent impulses reaching the center may determine the state of this center and the character of the reflexes in which it takes part.

EXPERIMENTAL METHOD

In order to solve the first task we selected cats in which a chemical stimulation of the receptors of the pericardium of any intensity caused only pressor or depressor reactions. As an indicator of the state of the vasomotor center we used the reaction of the cardiovascular system to compression of the left common carotid artery (the right was ligated since the cannula for recording the blood pressure was inserted into it). For the second task we used 7 cats whose carotid sinus zones had been denervated 30-40 days before the acute experiment.

Denervation was carried out by means of bilateral extirpation of all the tissues situated at the bifurcation of the common carotid artery into the external and internal, the adventitia of the vessels was removed and the arteries painted with a 5% solution of phenol. The methods of chemical stimulation of the receptors of the pericardium and of recording the blood pressure and respiration were described in Communication I.

EXPERIMENTAL RESULTS

In the experiments in which stimulation of the pericardial receptors with nicotine in all the concentrations used caused an increase only in the blood pressure, transient compression of the common carotid artery raised the blood pressure very sharply (Fig. 1, a). This reaction was especially obvious if both pressor and depressor reflexes were observed. If in some experiments nicotine caused depressor reaction, the reflex to compression of the carotid artery was then markedly weakened or even distorted (Fig. 1, b).

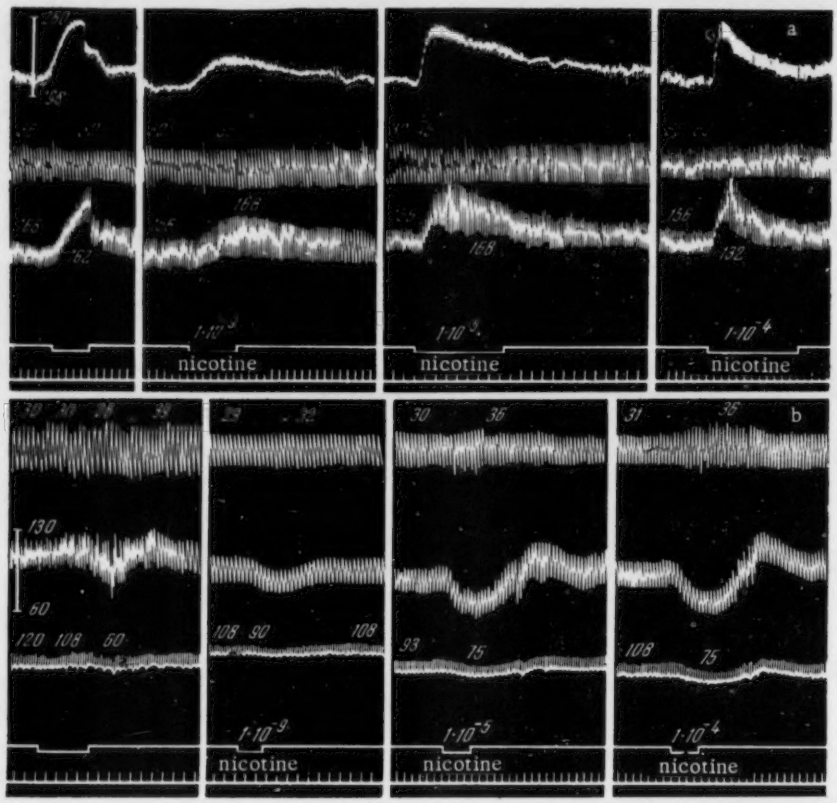

Fig. 1. Comparison of the reactions to compression of the left common carotid artery in cats reacting to stimulation of the pericardial receptors with nicotine in any concentration by either pressor (a) or depressor (b) changes in the blood pressure only (experiment on cats under urethane anesthesia).
Significance of the curves (from above downwards): on the series a kymograms: blood pressure (mercury manometer), respiration, blood pressure (membrane manometer), stimulation marker, time marker (5 seconds); on the series b kymograms: respectively respiration, blood pressure (mercury manometer), remainder as in the series a kymograms. The figures along the respiration curve indicate the respiration rate at that particular moment, and the figures along the membrane manometer curve – the rate of the heart (both calculated per minute); compression of the carotid artery, see the stimulation marker in the first kymograms on the left.

From a comparison of these facts it can be concluded that in the first case (see Fig. 1, a) the state of the vasomotor center brought about predominance and intensification of the pressor reactions, whereas in the second case (see Fig. 1, b) the pressor reflexes in which the vasomotor center took part, irrespective of whether caused by

chemical stimulation of the pericardial receptors or by compression of the carotid artery, were effected with difficulty, i.e. inhibition was dominant in the vasomotor center.

The state of the vasomotor center thus affects equally the reflexes caused by stimulation of the carotid sinus and the pericardial reflexogenic zones.

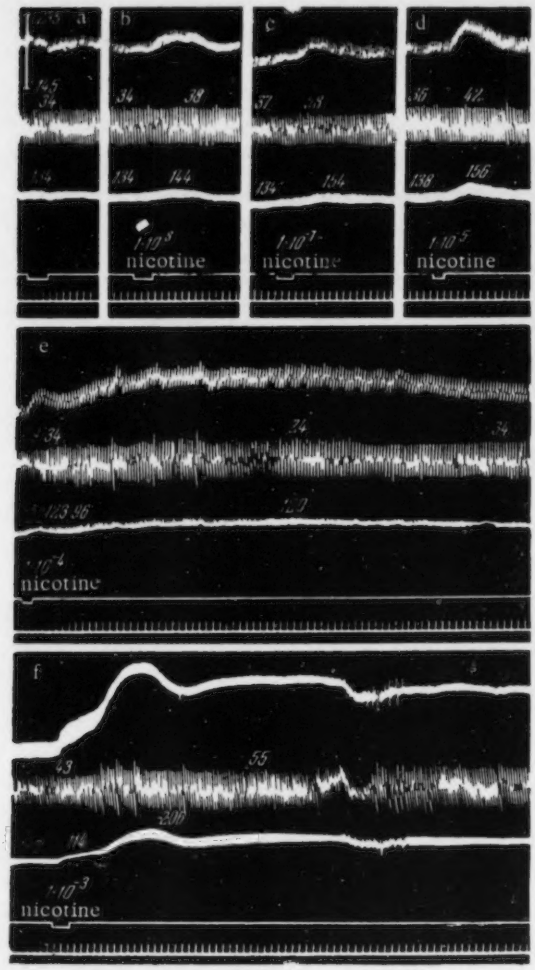

Fig. 2. The character of the reactions of the cardiovascular system and respiration to stimulation of the pericardium with nicotine in various concentrations (b-f) in cats whose carotid sinus zones had been denervated 40 days before the experiment.
On kymogram a – compression of the left carotid artery. The order of the tracins and the symbols are the same as in Fig. 1,a.

Exclusion of impulses from these zones causes similar changes in the blood pressure (see Communication II) and evidently identical changes in the center, brought about in both cases by a reduction in the number of afferent impulses reaching it. This provides good grounds for the attempt to influence the character of the reflexes caused by stimulation of the pericardial receptors by means of a reduction in the afferent impulses reaching the vasomotor center from the carotid sinus zones.

In all the experiments on cata with previous denervation of the carotid sinuses a chemical stimulus of any intensity applied to the pericardial receptors raised the blood pressure (Fig. 2). This indicates that the afferent impulses arising during stimulation do not themselves determine the character of the reflex reaction, but acquire some degree of importance which depends on the number of impulses reaching the vasomotor center not only from the stimulated reflexogenic zone but from others as well.

By artificially changing the number of impulses we make it possible to correct the vasomotor reactions. In this particular case, for instance, in animals whose vasomotor center received no impulses for a long time from the mechanoreceptors of the carotid sinuses, a raised blood pressure was observed in response to strong stimuli, which caused a fall in blood pressure in the majority of normal animals. In both the 25% of animals with intact carotid sinuses (see Communication I) in which only pressor reactions were observed to chemical stimulation of the pericardium, and in the animals undergoing the preliminary operation, the state of the vasomotor center favored predominance of the pressor reactions.

However, the magnitude of the pressor reactions of the blood pressure in the animals of these two groups were altered to a different extent as the strength of stimulation increased. This can be seen from a comparison of Figs. 1, a and 2. If in the cats with intact carotid sinuses the reactions to stimulation of the pericardial receptors increased in proportion to the intensity of stimulation and reached a maximum at only comparatively small concentrations of the stimulus (nicotine $1 \cdot 10^{-5}$), in the cats with denervation of the carotid sinus zones the reactions to weak and average concentrations of the stimulus ($1 \cdot 10^{-8}$ - $1 \cdot 10^{-4}$; Fig. 2, b-e) hardly differed at all from each other and were expressed as a small rise in the blood pressure. The reaction to the highest concentration of nicotine, however, was extremely intense, and was greater than the pressor reactions to the same stimulation of the pericardial receptors even in cats with intact buffer

nerves, in which pressor reactions only were observed.

Special experiments on animals of the latter group provide an explanation of these differences mentioned. If in these cats the reactions to compression of one of the carotid arteries ("carotid reflex") is recorded on a background of the pressor reactions caused by chemical stimulation of the pericardial receptors, then the change in the character of the carotid reflex will depend on the intensity of the pericardial reflex. In Fig. 3 it can be seen that at the peak of the small rise in blood pressure cuased by a chemical stimulus of average intensity (nicotine $-1 \cdot 10^{-5}$) applied to the pericardial receptors, the "carotid reflex" which is clearly marked anyhow in these animals increases still further; as a result the total effect of the two forms of stimulation exceeds the arithmetic sum of the reactions to each of them individually (see Fig. 3, a).

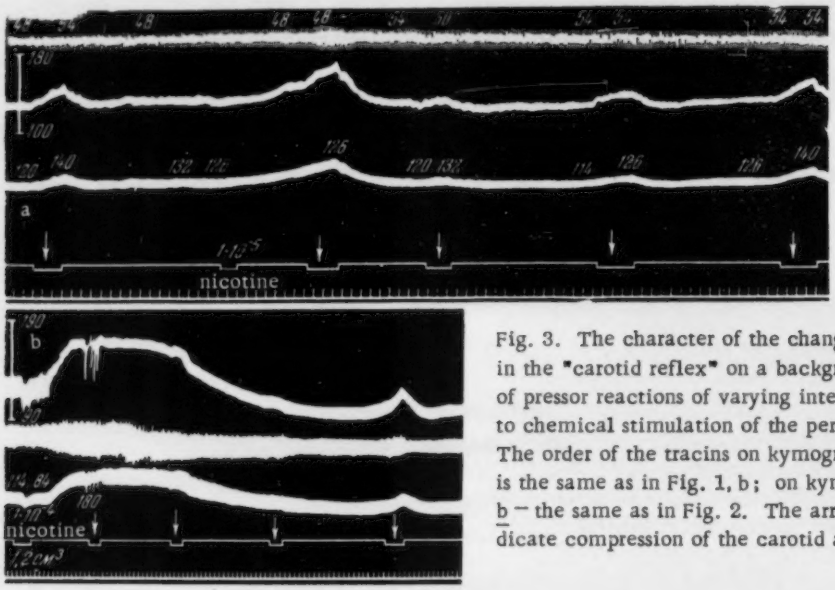

Fig. 3. The character of the changes in the "carotid reflex" on a background of pressor reactions of varying intensity to chemical stimulation of the pericardium. The order of the tracins on kymogram a is the same as in Fig. 1, b; on kymogram b – the same as in Fig. 2. The arrows indicate compression of the carotid artery.

At the peak of a great increase in the blood pressure, caused by strong stimulation of the same receptors (nicotine $-1 \cdot 10^{-3}$), the "carotid reflex" is distorted, lowering the level of the blood pressure which had been attained beforehand, which weakens the effect of stimulation of the pericardial receptors (see Fig. 3, b).

On the basis of these experiments it may evidently be postulated that in animals with normally functioning carotid sinus reflexogenic zones, these zones exert their buffering or compensatory function not only in the form of signals reporting severe changes in pressure in the carotid sinus, but also as a result of the constant influence of impulses flowing from the receptors of this zone on the state of the vasomotor center; the character of these influences is opposite, depending on the number of afferent impulses reaching the vasomotor center from the other reflexogenic zones. Summating with them, impulses from the carotid sinus zones intensify the excitation of the vasomotor center during reception of moderate impulses along other nerves, but partially inhibit it when impulses from other sources are strong. In cases when impulses from the carotid sinus receptors are absent, reactions to moderate stimulation of other zones are weakened but reactions to strong stimulation are abnormally intensified (see Fig. 2). The dual nature of the influence of the reflexogenic zones of the thorax on the blood pressure has been pointed out previously by Hering [12], V. V. Parin [3, 4] and Wang and Borison [13], who have shown that the depressor effect of electrical stimulation of the carotid sinus and aortic nerves, exactly like the analogous effect of adequate stimulation of the pressure receptors of the carotid sinus or the pulmonary vessels, is increased both after denervation of the other reflexogenic zones of the thorax and during simultaneous stimulation of these zones or of the nerves issuing from them.

It is impossible to account for this duality from the standpoint of a qualitative specificity of afferent impulses (in this case depressor); it becomes understandable if it is accepted that a different number of impulses,

identical as signals, may lead to qualitatively opposite states of the center. This conclusion can be reached essentially from the facts described by Douglas and his co-workers [9, 10].

This is also suggested by our own findings [2]. If this is so, then the increased blood pressure in response to compression of the carotid arteries must be regarded not as the consequence of weakening of the tonic inhibitory afferent influences on the vasomotor center but as the result of the action of this center of a different (smaller than normal) number of afferent impulses, causing excitation of the center and, it seems, having a different meaning as a signal.

In such a case the raised arterial pressure in response to exclusion of afferent impulses from any reflexogenic zone in the thorax must be regarded as the result of a diminution of the total sum of afferent impulses reaching the vasomotor center down to the number causing greater excitation of the center than at the level of reception of impulses which is normally observed. This is indirectly shown by the fact that in animals with denervation of the carotid sinuses the pressor effect of strong stimulation of the pericardial receptors is more pronounced than in normal animals. However, the individual component parts of the vasomotor center have their own special features, due to whatever reflexogenic zones supply the afferent fibers to the particular group of nerve cells; or more exactly, either constant impulses travel to these cells (as, for example, from the carotid sinues or the arch of the aorta) or they are episodic, arising in response to extraneous stimuli (for example from the epicardial receptors during chemical stimulation).

Under these circumstances a certain "normal" state of the vasomotor center is due to definite interaction of its component groups of nerve cells, in which excitation of the center as a whole, and consequently an increase in blood pressure, may rise only to a particular level; above this level, excitation is transformed into inhibition of the center as a result of further increase in afferent impulses, which leads to a compensatory fall in the blood pressure. However, in animals such as rabbits, in association with the peculiarities of their ecology, the normal state of their center is such that any additional stimulation inhibits it and causes a fall in the blood pressure [7, 8]. In these animals also, under suitable conditions of anesthesia, pressor reactions to weak stimulation of the pericardium can be observed [2].

It is characteristic that denervation of the carotid sinus, which alters the character of the vascular component of the reactions under study, hardly affects the character of the changes in the cardiac component. In Fig. 2, d, e, it can be seen that whereas strong stimulation of the pericardium leads to an increase in the blood pressure, the rate of the heart is at first not increased but decreased. This is understandable, since inhibition of the heart is not a feature of the medullary centers but of the structures of the heart itself [6], and so by altering the number of impulses to the medulla we destroy the conditions necessary for inhibition of the vasomotor center but not the conditions determining inhibition of the heart. It is true that during the strongest stimulation (see Fig. 2, e) inhibition of the heart is replaced, during growth of the vascular reaction, by a considerable increase in its rate, which may be regarded as the result of inhibition of the bulbar center of the vagus nerves; but this is presumably the consequence of wide irradiation of excitation in the medulla, manifestations of which may be seen also in the unusually prolonged vascular reaction, and in the appearance during its course of vomiting movements (see Fig. 2, e) and of periodic respiration (see Fig. 2, d, e) and even of inhibition of respiration (see Fig. 2, d). We never observed these latter phenomena in animals with intact carotid sinuses; in these animals stimulation of the pericardial receptors always led to stimulation of respiration, which we explained by the small number of connections between the pericardium and the respiratory center. However, P. A. Spiro [5] showed that as the afferent influences are strengthened, stimulation of respiration changes into inhibition, and with stimulation of intermediate strengths periodic respiration is observed. It may be postulated that in our experiments exclusion of the structures of the vasomotor center related to the carotid sinus reflex took place, as a result of which the conditions for the formation of the pericardial reflexes were destroyed. As a result the excitation irradiated to the adjacent areas of the vasomotor center and increased the duration of the vascular reaction.

SUMMARY

The stimulation of pericardial receptors by chemical substances in any concentration caused only pressor reactions in all cats with denervated carotid sinuses. However, these reactions could be observed only in 25% of the cats with the intact carotid sinuses. A cumulative effect of the separate action of these stimulants on the blood pressure was found in these 25% of the cases in clamping of the carotid artery on the background of a slight rise of the blood pressure caused by not very strong stimulation of the pericardial receptors. On the contrary, if the carotid

artery was clamped on the background of a considerable rise of the blood pressure caused by a strong stimulation of the pericardium these effects depressed each other reciprocally as a result of which a inversion in the carotid reflex was noted.

LITERATURE CITED

[1] N. E. Vvedenskii, Complete Collected Works, Vol. 2, Leningrad,(1951).*

[2] B. S. Kulaev, The Role of the Chemoreceptors of the Pericardium in the Regulation of the Ciruculation and of Respiration, Dissertation(Moscow,1954).*

[3] V. V. Parin, Transactions of the Sverdlovsk Medical Institute and the Research Institutes of the Regional Health Division, 15, pp. 3-87, Sverdlovsk, 1941.*

[4] Idem, The Role of the Pulmonary Vessels in the Reflex Regulation of the Circulation, Moscow, 1946.*

[5] P. A. Spiro, Novorossiisk University Papers, Vol. 15, No. 1, 1874.*

[6] M. G. Udel'nov, Structural and Functional Basis of the Inhibitory Influence of the Nervous System and the Nature of the Process of Inhibition in the Heart. Dissertation for Doctorate, Moscow 1955.*

[7] V. N. Chernigovskii, "The pericardial receptors." Neurohumoral Controls of the Activity of Organs and Tissues, pp. 54-79, Leningrad, 1941.*

[8] Idem, Afferent Systems of the Internal Organs, Kirov, 1943. *

[9] W. W. Douglas, I. R. Innes and H. W. Kosterlitz, J. Physiol. 1950, Vol. 111, p. 215-230.

[10] W. W. Douglas and W. Schaumann, Ibid. 1956, Vol. 132, p. 173-176.

[11] B.Folkow, Physiol. Rev. 1955, Vol. 35, p. 629-633.

[12] H. E. Hering, Die Karotissinusreflexe auf Herz and Gefässe, Dresden u. Leipzig, 1927.

[13] C. S. Wang and H. L. Borison, Am. J. Physiol. 1947, Vol 150, p. 712-721.

* In Russian.

THE BIOELECTRICAL ACTIVITY OF THE CORTEX AND SUBCORTEX OF DOGS DURING ELECTRICALLY INDUCED SLEEP

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The present work is a continuation of previous research on the study of the mechanism of action of an impulse current of low power and low frequency on the cerebral hemispheres [2].

EXPERIMENTAL METHOD

Experiments were carried out on dogs over long periods of time, after insertion of electrodes into the cortex and the optic thalamus. Both unipolar and bipolar tracings of the action currents, in the premotor, motor, temporal and parietal areas of the cortex and in the optic thalamus of the dogs were taken. The distance between the cortical electrodes for the bipolar tracing was 1 cm, and that between the deep electrodes was 2-3 mm. For the unipolar tracing of the bioelectrical activity of the cortex the indifferent electrode was placed in the nasal bones of the animal. The deep electrodes were of platinum wire, 120 μ in diameter, and coated except for the tip with insulating varnish. Two electrodes were inserted into the optic thalamus. For accurate localization of the electrodes in the animals, x-ray films of the head were taken. At the conclusion of the experiments the dogs were killed, the brain photographed and its cell structure examined. Each 5th section of a series of frontal sections (thickness of the section 20 μ) was stained by Nissl's method. Next the localization of the inserted electrodes in the preparations was determined microscopically. The electrograms of the optic thalamus (EOT) were recorded in all the dogs from the medial and ventrolateral groups of nuclei of the optic thalamus. The action currents were recorded on a six-channel ink-recording oscillograph. The dog was strapped in a Pavlov's apparatus. The investigation began with a tracing of the background electrical activity of the cortex (ECG) and of the thalamus. The impulse current was then switched on for 1 minute every 15-20 minutes (to avoid artefacts) and at this time the action currents of the sections of the brain under examination were recorded. The length of the sessions of electrically induced sleep was 2 hours. 20 sessions were carried out with each animal. At the conclusion of the course of electrically induced sleep the action currents of the cortex and thalamus were recorded in these dogs for a further period of 3-4 weeks. The impulse current used was of different frequencies – 6, 10, 12 and 18 impulses/sec. Current was supplied to both the cortical electrodes and the electrodes inserted into the thalamus. In four animals electrically induced sleep was carried out at first with a current of one frequency, for example at 10 impulses/sec and a few months later at another frequency, for example 18 impulses/sec. In all 220 sessions of electrically induced sleep were carried out on 7 dogs.

EXPERIMENTAL RESULTS

One dog (Polkan) fell asleep during the first session of electrically induced sleep. In the dog Pushok drowsiness appeared at the end of the first session, and a profound sleep developed during the second session. Three dogs (Ryzhik, Arabka and Belka) slept under the action of the impulse current after the second and fourth sessions. The dog Druzhok began to sleep only after the fifth session, but the dog Volchok did not sleep at all under the influence of the impulse current, but stayed quietly in the apparatus throughout its whole period of action. Four of

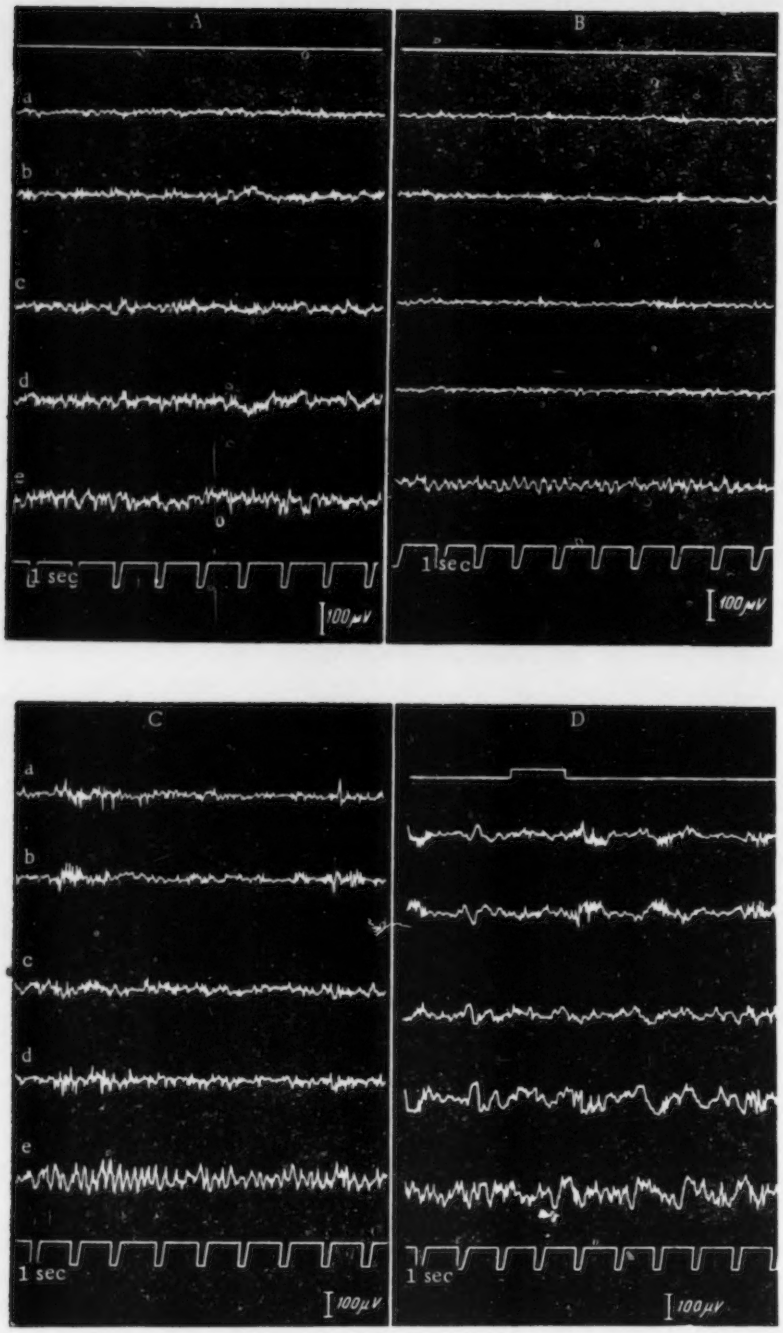

Fig. 1. Electrocorticogram of the dog Polkan recorded during the first session of electrically induced sleep. Bipolar tracing.
a, b, c, d) Electrocorticograms of the motor, sensory, temporal and parietal areas of the cortex; e) EOT; A) initial background electrical activity; B, C, D) tracings made 15, 30 minutes and 1 hour after the current was switched on.

these animals, subjected after 2-3 months to a second course of action of the impulse current at a different frequency, showed almost the same behavior at the sessions of electrically induced sleep. Polkan and Belka fell asleep at the second session, Druzhok at the third and fourth sessions simply dozed and actually slept only at the fifth session, and Volchok did not sleep at all, although he was somewhat drowsy.

Usually definite changes in the bioelectrical activity of the brain are observed in the dog even in the first session of electrically induced sleep. They are shown by the appearance of rhythmic electrical oscillations in the thalamus, by an increase in the amplitude and synchrony of the rapid oscillations on the ECG and a subsequent fall in the amplitude of the action potentials of the cortex to 10-15 μv (Fig. 1, B). With continued action of the impulse current the amplitude of the rhythmic waves in the thalamus increases from 50-60 to 100-120 μv. Bursts of rhythmic waves with a frequency of about 14 per second (α-like waves) appear on the ECG, and these settle down into slow waves with a frequency of 5-7 per second (Fig. 1, c). Later on there is a slowing of the low-frequency processes in the cortex and an increase in their amplitude. The rhythmic oscillations in the thalamus are replaced by large, slow waves with a frequency of about 1 per second and an amplitude reaching 130 μv (Fig. 1, d). The animal goes to sleep strapped up. However any external stimulation – a sound, a flick of the thumb, a slight touch, the entry of the experimenter into the chamber – immediately causes it to awaken, with disappearance from the electrocorticogram (ECG) of the above-mentioned bursts of waves with a frequency of 14 per second and with restoration to the electrogram of the optic thalamus (EOT) of its rhythmic oscillations with a frequency of 4 per second. With deepening of the sleep the amplitude of the slow waves in the cortex grows considerably to reach 100 μv, but their frequency falls to 1-1.5 oscillations per second. Slow waves with an amplitude of 120--130 μv dominate the EOT. With sleep of this depth a sound does not always cause awakening of the animal. In some cases even electrical stimulation of the skin causes awakening of the animal, disappearance of the slow waves from the ECG and appearance of the characteristic rhythmic activity (4-5 per second) on the EOT only after repeated application (slightly over the threshold value). Only 2-3 minutes afterwards slow waves reappear in the cortex, followed by the thalamus. The sleep usually continues after the current is switched off. Its duration varies – from 20 minutes to 1 hour. In the cortex at this period there is seen a gradual fall in the amplitude of the slow waves and the appearance of α-like waves, whereas in the thalamus waves appear with a rhythm of 4-5 per second.

It must be pointed out that in all the dogs except Volchok and Belka, in some cases after 10-14 sessions it was possible to discern a conditioned reflex sleep, which appeared on the ECG in the form of changes identical with those during electrically induced sleep. Conditioned reflex sleep developed when the animal was placed in the apparatus and the electrodes connected up without the current being switched on. It lasted for different lengths of time – from a few minutes to 30-40 minutes.

In those cases where the animal did not go to sleep in the sessions of electrically induced sleep (for example the dog Volchok), the ECG showed a considerable fall in the amplitude of the waves, particularly in the anterior divisions of the cortex, with the appearance of bursts of α-like waves with an amplitude of 60-70 μv. On the EOT at this time were observed synchronous rhythmic waves with a frequency of 4-5 per second.

It shoul be mentioned that the rhythm of 4-5 waves per second which appears at the end of the first or during the second session is maintained in the thalamus for a longer time. Usually at the third or fourth session it is the dominant process, overshadowing the background EOT. At the conclusion of 20 sessions of electrically induced sleep, the rhythm of 4-5 waves per second continues to be recorded in the thalamus of the dog for 2-3 weeks, and the electrical activity of the brain returns only gradually to its initial background level.

Rhythmic oscillations with a frequency of 4-5 per second appear in the thalamus of the dog in all cases in which electrically induced sleep is carried out, irrespective of the frequency of the impulse current used. Consequently, irrespective of the frequency of the external stimulus, the brain tissue reacts to it at its own optimal rhythm of excitation.

The slowing of the frequency of the dominant rhythm of the cortical structures in the course of development of electrically induced sleep demonstrates a fall in the lability of the cortex. In spite of the fact that the subcortical formations are in the zone of greatest intensity of action of the current (as pointed out by N. M. Liventsev et al. [1]), the development and deepening of the state of inhibition (slowing of the frequency of the dominant rhythm) is observed primarily in the cerebral cortex. In the conditions of the present investigations, when the impulse current was supplied directly to the electrodes inserted into the brain tissue, the role of tactile receptors

was eliminated. Consequently the changes in the bioelectrical activity of the brain were due to the direct action of the impulse current on the nerve cells of the cerebral hemispheres.

SUMMARY

Impulse current of low intensity and frequency has a weak parabiotic effect on cerebral hemispheres. The functional changes appearing under the effect of impulse current are retained in the central nervous system after cessation of its action. The longer was the action of the current the more prolonged the retention of the functional changes in the central nervous system. The majority of animals fall asleep due to the action of impulse current. The depth of sleep varies in animals depending on the individual peculiarities of the function of the central nervous system.

LITERATURE CITED

[1] V. A. Giliarovskii, N. M. Liventsev, Iu. E. Segal' and Z. A. Kirillova, Electrically Induced Sleep, Moscow, 1953.*

[2] I. S. Robiner, Zhur. Vysshei Nerv. Deiatel., 6, 146-156 (1956).

* In Russian.

THE RELATIONSHIP BETWEEN THE MOTOR REACTION OF THE PYLORIC PORTION OF THE STOMACH AND ITS FUNCTIONAL STATE

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Indications have recently appeared in the literature of the great importance of the functional state of the gastrointestinal tract in the performance of its motor reactions, and that the functional state of the gastrointestinal tract is connected with the period of work or rest. P. F. Bogach [1] was the first to establish that the strength and duration of the motor reactions of the small intestine vary in relation to its functional state. An increase in the excitation of the fundal portion of the stomach at the end of a period of rest was observed by E. M. Matrosova [6].

In view of the fact that the motor activity of the pyloric region differs from that of both the fundus of the stomach and the small intestine, we decided to investigate how the excitation of the pyloric section changes at different moments of a period of rest. Such an investigation is also of interest because the motor function of the pylorus has by no means received sufficient study.

EXPERIMENTAL METHOD

Experiments were carried out on 2 dogs 16-18 hours after feeding. In the dogs, Basov-Pavlov fistulas had been made in the fundal portion of the stomach. Through the fistulas and into the stomach were passed 2 rubber balloons, attached to nippled tubes. One balloon, 5 cm long, was attached to a short tube (2-3 cm), the other – with a length 2 or 5 cm depending on the aim of the investiation – was attached to a nippled tube 10-12 cm long. The small balloon (2 cm long) on the long nippled tube was used for registration of the basal motor activity of the pylorus, and the large balloon (5 cm long) for stimulation of the pylorus and at the same time for registering its motor activity Increase in the length of the balloon, and consequently in its volume, allowed reduction of that part of the pressure inside the balloon which resulted from the elastic tension of the walls of the balloon. The large balloon (5 cm long) on the short nipple was used for recording the motor activity of the fundal portion of the stomach. In some experiments, in order to fix the balloon in the pyloric region, we inserted a third balloon into the stomach, on a nippled tube 20-25 cm long. This balloon passed through into the duodenum. The base of the pyloric balloon was attached to the nippled tube of the duodenal balloon; the balloons were passed into the stomach one after the other. A special investigation showed that inflation of the duodenal balloon with 3-4 ml of air did not essentially modify the motor activity of the pylorus. In order to record the basal motor activity, 20-30 ml of air was introduced into the balloon in the fundus and 3-4 ml of air into the balloon in the pylorus.

For stimulation, from 15 to 60 ml of air was introduced into the balloon in the pylorus. The balloons were inflated with air by means of a special apparatus which permitted not only the introduction of a definite volume of air, but also a constant watch to be kept on the pressure inside the balloons. The motor activity of the pyloric and fundal portions of the stomach were recorded on the drum of a kymograph by means of water – air or air transmission and a Marey's capsule.

EXPERIMENTAL RESULTS

In all the experiments we observed periodicity of the motor activity of the pylorus, which coincided in time with the periodicity of the motor activity of the fundal portion of the stomach. Under these circumstances typical rest periods took place in the pylorus. These findings confirm the accuracy of I. A. Edel'man's [8] point of view of the presence of periodic motor activity in the pyloric segment. American workers [12], formerly supporting the opposite view, have lately been obliged to change their minds [9]. Thus, at the present time, thanks to the work of a number of authors [4, 7, 9, 10], the presence of periodic motor activity and typical rest periods in the pyloric portion of the stomach can be considered to be firmly established.

In the middle and end part of the working period, the amplitude of the pyloric contractions underwent a number of regular changes associated with the contractions of the fundal portion of the stomach. The amplitude of the pyloric contractions was least from 15-30 seconds after completion of the fundal wave; it then gradually increased and reached its maximum at the moment of development of the next fundal wave, or slightly afterwards. The next 1 or 2 pyloric contractions remained at the maximum amplitude, after which their amplitude sharply diminished and the whole cycle of changes in the amplitude started afresh (Fig. 1). In the initial part of the period of work, when the fundal waves had not quite reached their normal size, these regularities were less clearly seen. At the end of the period of work, the variations in amplitude became particularly large.

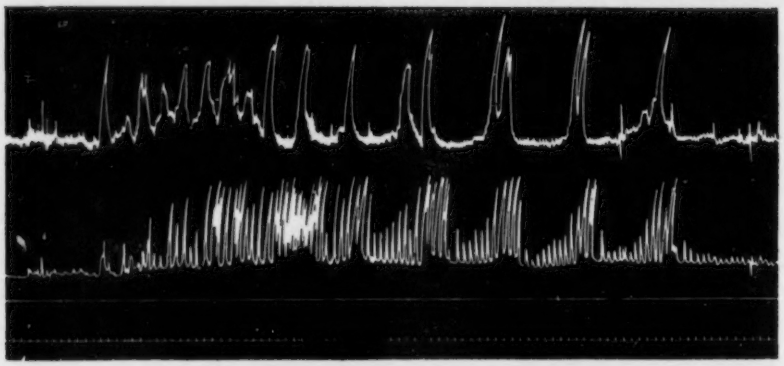

Fig. 1. Period of work in the fundal and pyloric portions of the stomach. (At the beginning of the period of work the pyloric balloon several times slipped through into the duodenum).
Significance of the curves (from above downwards): motor activity of the fundus, motor activity of the pylorus, stimulation marker, time marker (17 seconds).

On inflation of the balloon in the pyloric segment of the stomach to 20 ml during a period of work, the amplitude of the pyloric contractions was considerable increased, although the character of the variations in amplitude remained unchanged (Fig. 2, first half). On increasing the stimulating volume to 30 ml, the amplitude of the less powerful contractions continued to grow. The contractions of the pyloric segment became more even, but right at the end of the period of work the variations in the amplitude were restored.

Further distension of the stimulating balloon with air to 50-60 ml led to a diminution of the amplitude of the pyloric contractions (see Fig. 2, second half). For example, if the pyloric balloon contained 15-20 ml of air, the pressure inside the balloon during maximum contraction reached 80-90 mm of mercury, then after introduction of a further 40 ml of air it did not rise during these contractions higher than 50-60 mm of mercury. In spite of the fact that this volume was evidently excessive, when the stimulus was applied we observed no restlessness on the part of the dog. Gradual escape of air from the pyloric balloon enabled all the above-mentioned phenomena to be observed in the opposite order. The depressing influence of a strong mechanical stimulus on the motor activity of the pylorus during experiments of short duration on dogs was pointed out by McCrea and McSwiney [11].

During inflation of the pyloric balloon with 20-40 ml of air in the course of a period of rest, the appearance

of a series of 2-4 contractions of the pylorus was noted, whose amplitude rapidly fell. A similar picture during mechanical stimulation of the pyloric portion of the stomach was observed by V. F. Mostun [7]. The contractions of the pyloric portion were always accompanied by motor activity of the duodenum, and often – of the fundal portion of the stomach. As the moment of inflation of the pyloric balloon drew closer to the next working period, the reaction of the pyloric portion gradually strengthened. The contractions arising during inflation of the pyloric balloon 10-15 minutes before the start of the next period of work could be directly transformed into the contractions of the period of work. The time interval between the onset of inflation of the pyloric balloon to 20-30 ml and the onset of the period of work was not constant in the whole of the gastrointestinal tract and depended on the moment of the rest period at which inflation began. Increase in the stimulating volume to 50-60 ml strengthened the reaction up to and including cessation of the rest periods in the pylorus. The contractions occurring differed from the usual "hunger" contractions by their smaller size and also by the complete absence of the characteristic variations in amplitude. Later on the amplitude of these contractions gradually increased and at the time of appearance of the next period of work it had become maximal. We judged the onset of the period of work by the appearance of type A contractions (according to S. V. Anichkov) in the fundal portion of the stomach; in corresponding fashion the end of the period of work was determined. In spite of the quite considerable motor activity of of the pyloric portion, the fundal portion of the stomach sometimes remained at rest.

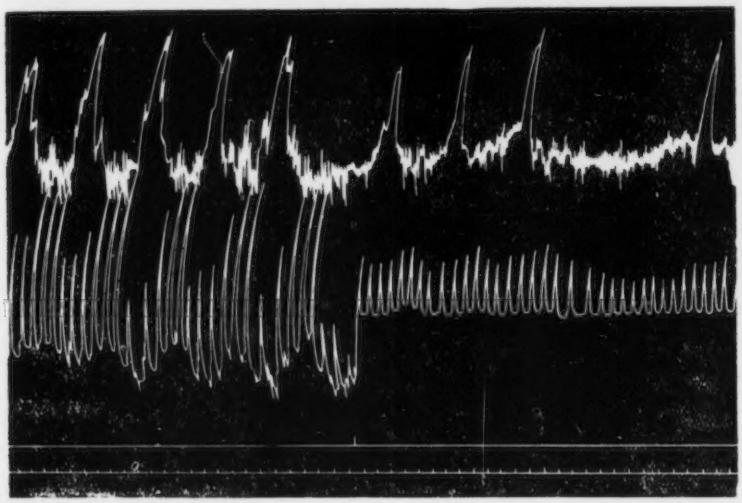

Fig. 2. The effect of strong inflation of the pylorus on its motor activity. (In the first place 15 ml of air was introduced into the pyloric balloon; at the moment indicated by a stroke on the stimulation marker an additional 35 ml of air was introduced into the pyloric balloon). Significance of the curves as in Fig. 1.

The absence of contractions of the fundus at a time when quite strong contractions were taking place in the pylorus demonstrates the relative independence of the motor function of the fundus from that of the pylorus. On the other hand, inflation of the balloon in the fundus always caused the appearance of characteristic motor activity in the pylorus at the same time as the contractions of the fundus [5]. Analysis of the curves of basal motor activity of the pyloric and fundal portions also indicates that the pyloric contractions can be changed by the influence of motor phenomena in the fundal portion of the stomach. In all this we see the manifestation of the polarity of the stomach – a characteristic property of the gastrointestinal tract which allows movement of the chyme in a caudal direction.

In some experiments the pyloric balloon, filled with 30 ml of air in the course of the period of work, remained inflated throughout the whole of the succeeding period of rest. Under these circumstances we were able

to observe how the excitation of the pylorus changes from the very beginning to the end of the rest period. A series of portions of the curve of pyloric contractions taken at different moments after the onset of the rest period in the fundus is illustrated in Fig. 3. In this experiment the pyloric balloon was inflated with 30 ml of air. As seen in Fig. 3, the least excitation in the pylorus was at the beginning of the period of rest; later the excitation of this portion of the stomach gradually increased and reached a maximum at the moment of appearance of type A contractions in the fundus. An increase in the excitation of the pyloric portion of the stomach was reflected not only in an increase of the amplitude, but also in a change in the frequency of the contractions. In one of the experiments inflation of the pyloric balloon to 40 ml in the first half of the period of rest caused contractions with a frequency of 3 per minute, but at the end of this period – a frequency of 5 per minute.

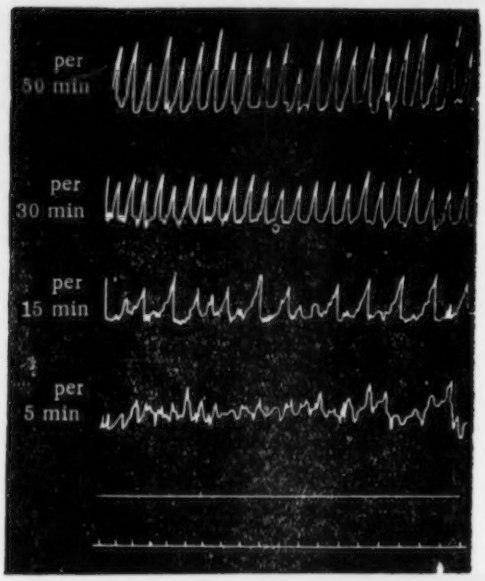

Fig. 3. Motor activity of the pylorus during inflation of the pyloric balloon with 30 ml of air. (Areas of the curve are shown which demonstrate the motor activity of the pylorus at various intervals of time after the onset of a rest period in the fundus).
Significance of the curves (from above downwards): first, second, third and fourth curves – tracings of the motor activity of the pylorus; fifth – stimulation marker; sixth – time marker (17 seconds).

Prolonged inflation of the balloon in the pylorus to 50 ml enabled the discovery of one more detail in the change of excitation of the pyloric portion of the stomach. During inflation of the balloon to the volume mentioned, the motor activity of the pylorus was more marked during the first 5-7 minutes after the onset of the period of rest than during the following 10 minutes. Later on the motor phenomena in the pyloric portion of the stomach developed exactly as described above. When we used smaller stimulating volumes, the contractions in the first third of the rest period were completely absent.

During inflation of the balloon in the pylorus, the periods of work in the fundus were lengthened, and at times they were from $1\frac{1}{2}$ to $2\frac{1}{2}$ times as long as control periods of work; at the same time the duration of the rest periods was somewhat shorter.

The experimental results show that the characteristic variations in the amplitude of the pyloric contractions during periodic motor activity depend on the motor phenomena in the fundus, since the pyloric contractions caused by inflation of the fundus [5] or by direct mechanical stimulation of the pylorus do not show these variations. Our experimental findings demonstrate that the motor reactions of the pyloric portion of the stomach during stimulation of this region during a rest period are intensified as the moment of stimulation draws nearer to the end of the period of rest. A gradual increase in the excitation of the pylorus is observed at the end of its rest period. These findings of the change in the magnitude of the motor reactions and the excitation of the pylorus at different moments of the period of rest are in full agreement with the results obtained by P. G. Bogach [1, 2, 3] from a study of the response reactions and the excitation of the small intestine.

SUMMARY

The motor function of pylorus and the effect of mechanical stimulation on the contraction of this portion of the stomach were studied in chronic experiments on dogs. Periodical motor activity in pylorus is characteristic for an empty stomach. Weak and average mechanical stimulation intensify the motor function of the pylorus, while strong ones depress it. In stimulation of the pylorus during the period of rest its motor reactions in response to stimulation are gradually intensified with the approach of the terminal phase of the period of rest.

LITERATURE CITED

[1] P. G. Bogach, Biull. Eksptl. Biol. i Med. 40, 7, 16-20 (1955).

[2] Idem, Kiev University Scientific Papers, Physiological Collection, No. 9, 15, 12, 55-63, (1956).*

[3] Idem, Biull. Eksptl. Biol. i Med. 43, 5, 31-36 (1957).**

[4] D. Kh. Golshtein, Russk. Fiziol. Zhur. 9, 1, 148-151 (1926).

[5] S. D. Groisman, Proceedings of a Scientific Meeting on the Problems of the Physiology and Pathology of Digestion, pp. 57-59, Tartu, 1957.*

[6] E. M. Matrosova, Ibid. pp. 160-161.*

[7] V. F. Mostun, Interrelations Between the Motor Activity of the Different Areas of the Alimentary Tract During Starvation. Dissertation, Moscow, (1956).*

[8] I. A. Edel'man, Movements of the Stomach and the Passage of its Contents into the Intestine, St. Petersburg (1906).*

[9] D. A. Brody, J. M. Werle and I. Mechan et. al., Am. J. Physiol. 1940, Vol. 130, p. 791-803.

[10] C. F. Code, N. C. Hightower and C. G. Morlock, Am. J. Med. 1952, Vol. 13, p. 328-351.

[11] E. D. McCrea and B. A. McSwiney, A. J. Physiol. 1926, Vol. 61, p. 28-34.

[12] I. Mechan and J. P. Quigley, Am. J. Physiol. 1938, Vol. 121 p. 350-357.

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* In Russian.

** Original Russian pagination. See C. B. Translation.

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE MOTOR FUNCTION OF AN ISOLATED SEGMENT OF SMALL INTESTINE IN DOGS WITH ACTURE RADIATION SICKNESS

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In radiation sickness the condition of the alimentary tract undergoes a number of disturbances, and for this reason the study of the motor function of the small intestine in radiation sickness arouses particular interest. At the same time this problem is rarely discussed in the literature. There are only isolated investigations on small laboratory animals. Conard [7], for instance, demonstrated an increase in the motor function of the intestine of rats exposed to a dose of only 100 r; under these conditions there is an increase in the muscle tone and in the amplitude of the contractions. An acceleration of the passage of the contents through the gastrointestinal tract in rats was reported by Goodman, Lewis and Schuck [8]

EXPERIMENTAL METHOD

Our experiments were carried out on 3 dogs on which the Thiry-Vella operation had been performed and an area of the small intestine 20 cm long isolated in the region of the lower part of the duodenum and the proximal jejunum. The movements of this isolated segment were recorded for 4 hours every other day by means of a pneumatic system with a rubber balloon, the pressure inside which was constant and amounted to 20 mm of mercury. In normal conditions before irradiation the motor activity of the isolated intestine was studied in dogs with an empty stomach and also after a meat meal (200 g).

EXPERIMENTAL RESULTS

In the animals before feeding we observed periodic contractions of the isolated intestine, which were particularly well marked in the dog Iula. In the remaining dogs – Pirat and Anakonda – the rest period was associated with smaller contractions of the loop of intestine. The periods of activity of the intestine in the hungry dogs lasted 2-3 minutes. The size of the contractions at the height of the period of activity was usually equivalent to 15-20 mm of mercury. The number of contractions per minute was constant and amounted to 14-16. The tone of the isolated area of intestine changed in an undulating manner: during a period of work the tone increased and at rest it fell to a minimum. The greatest value of the tone, which was observed when the contractions were maximal, was 2-6 mm of mercury. The periods of rest lasted 1-2 minutes. In some experiments we were unable to determine the periodicity of the movements of the isolated intestine.

The results which we obtained are in full agreement with the experimental findings of L. S. Girshberg [2] and L. G. Voronin [1].

When the dogs were feeding on meat, which was given immediately before the experiment, the isolated intestine responded to this simulus by a slight increase in the motor activity. Under these circumstances the period of activity was lengthened to 4-6 minutes, and the period of rest was shortened.

In order to obtain experimentally an increase in the motor activity of the isolated intestine, the dog was injected subcutaneously with proserin (prostigmin methylsulfate). Proserin was given to Pirat in a dose of 0.1 mg. Clinically this dose caused hardly any change. The motor activity of the intestine was intensified 6 minutes after the injection of proserin. Anakonda and Iula were injected with the same dose of proserin – 0.1 mg. In all cases we observed an increase in the motor function of the isolated intestine.

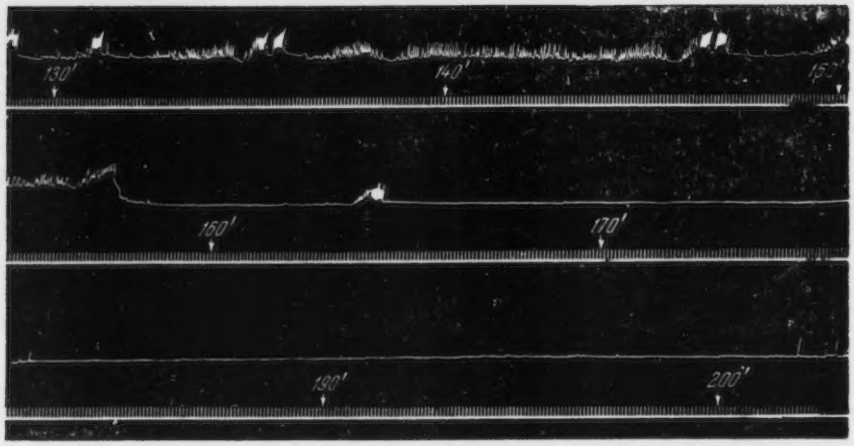

Fig. 1. Motor activity of the isolated intestine in the dog Pirat on the first day after irradiation with x-rays.

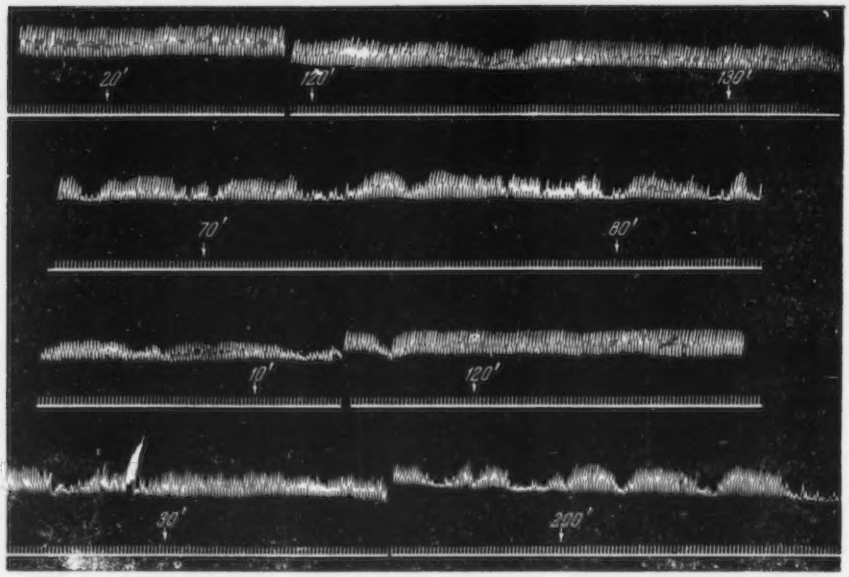

Fig. 2. Movements of the isolated intestine in the dog Anakonda on the 1st, 2nd, 8th and 14th day after irradiation.

In order to produce an artificial reduction in the motor function of the isolated intestine the dogs were injected subcutaneously with atropine in a dose of 1 ml of a 1 : 1000 solution. The injection of atropine to the dog Pirat was followed by prolonged rest of the isolated loop of intestine, which came on after 2 minutes 20 seconds. The suppression of motor activity of the intestine lasted 103 minutes 40 seconds. It must be pointed out that under these conditions we were unable to observe complete rest. At definite intervals of time we could observe isolated contractions of the loop of intestine. Injection of atropine to Anakonda and Iula also gave rise to depression of the motor function of the isolated loop of small intestine. These findings are also in agreement with the research of L. S. Girshberg [2] and L. G. Voronin [1].

Acute radiation sickness was produced in the dogs by total irradiation with x-rays (in a dose of 400 r). Three

dogs were irradiated. In Pirat the disease took a severe course and the animal died on the 22nd day after irradiation; in Anakonda and Iula the acute radiation sickness developed in a slight form.

We give below the results of investigations with the individual animals.

The dog Pirat. Tracing of the movement of the isolated loop of small intestine began 50 minutes after irradiation. At once a significant fall in the motor activity of the isolated intestine could be observed. The number of contractions per minute remained normal, but their strength fell to 12 mm of mercury. Fifty-eight minutes after irradiation the strength of the contractions had reached the level of 5 mm of mercury, and after 85 minutes it had fallen to 2 mm of mercury. Side by side with the depression of peristaltic movements spasms were recorded, lasting for 3-4 minutes. On the 150th minute the animal was observed to vomit. Two minutes after vomiting, the strength of the contractions increased; at the same time the tone of the intestinal musculature rose considerably, in some cases to 24 mm of mercury. Beginning on the 208th minute after irradiation, and continuing to the end of the experiment, a prolonged and almost complete rest of the isolated intestine developed; very occasionally isolated, single contractions with a magnitude of 2mm of mercury could be observed.

Thus, on the first day after irradiation the motor function of the isolated intestine was sharply inhibited (Fig. 1).

On the 2nd day after irradiation, in the first hour of the experiment the motor activity of the intestine was still depressed, but to a far lesser degree. The strength of the contractions reached 12 mm of mercury, but their frequency had fallen to 10 per minutes. In the second hour a violent motor activity was observed in the isolated intestine, with a contraction strength of 27 mm of mercury and an increase of tone to 28 mm of mercury. This sharply increased motor activity terminated in vomiting, after which it was considerably inhibited. The strength of the contractions reached at this moment 8 mm of mercury, and their rate fell to 4 per minute. This condition lasted until the 108th minute from the beginning of the experiment, after which quite prolonged periods of activity with contractions of varying strength were noted. The periods of contraction lasted for 5 minutes and sometimes even longer.

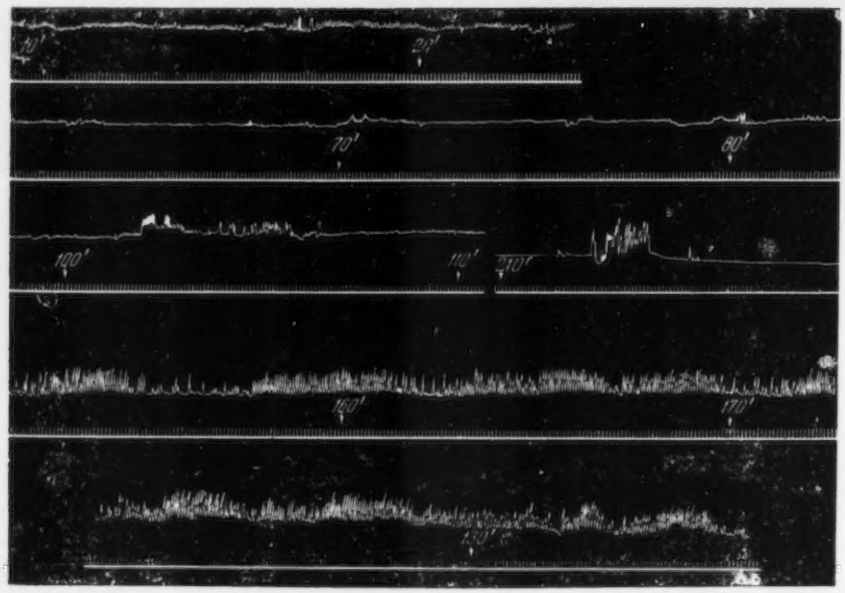

Fig. 3. Motor activity of the isolated intestine in the dog Iula on the 1st day (upper 3 curves) and on the 3rd and 5th days after irradiation.

On the 4th day after irradiation the motor activity was almost normal, i.e. the strength of the contractions was equal to 20 mm of mercury and their frequency 15 per minutes. On the 5th day the motor function of the

isolated intestine was slightly weaker; the maximum strength of the contractions was 16 mm of mercury but their rate had fallen to 14 per minute. On the 9th day after irradiation the contractions were uncoordinated. On the 14th day very powerful motor activity of the isolated loop of intestine was observed: the work periods were lengthened to 4-5 minutes, the contraction strength reached 30-35 mm of mercury, and their frequency was within normal limits – 15 per minute; the tone of the isolated intestine was raised to 14 mm of mercury. On the 17th day the tone was raised still further (27 mm of mercury), the number of contractions had fallen to 10 and their strength had increased to 48 mm of mercury. The increased motor activity of the isolated intestine continued until the 22nd day. At the beginning of the premortal period (22nd day after irradiation) the motor function diminished sharply; it then ceased altogether; the tone of the loop of intestine fell to zero. This condition lasted until the death of the animal.

The dog Anakonda. In the first hours after irradiation of the animal with x-rays an increase in the motor function of the isolated intestine could be detected. No changes in the intestinal tone were observed in the first 24 hours. The number of contractions per minute remained normal. The increase in the motor activity was the result of an increase in the strength of the intestinal contractions (to 32 mm of mercury), a lengthening of the periods of activity, and to an almost complete absence of periods of rest (Fig. 2).

The motor activity continued to remain high until the 14th day after irradiation. The strength of the constractions was 28 mm of mercury. On the 27th day the motor function of the isolated intestine fell to normal. On the 40th day a lowering of the motor activity could already be observed. The number of contractions per minute continued to remain normal, but their strength diminished to 4-8 mm of mercury. On the 48th day the strength of the intestinal contractions after the second hour of the experiment rose to 30 mm of mercury. On the 55th day the motor activity of the isolated intestine fell sharply; the number of contractions per minute was between 15 and 18, but their strength had fallen to 3-4 mm of mercury. On the 76th day after irradiation the motor function of the isolated loop of intestine was within normal limits.

The dog Iula. Only 20 minutes after irradiation of this animal with x-rays depression of the motor activity of the isolated area of intestine was observed. The contractions of the intestinal loop were uncoordinated. The strength of the intestinal contractions at this time was 4 mm of mercury, and 1 hour later – 2 mm of mercury. Side by side with the considerable reduction in the peristaltic movements, the tone of the isolated intestine was very often raised for a long time, usually to 4-6 mm of mercury.

It is in this manner that the intestinal spasms, so frequently observed in acute radiation sickness, can be explained. During vomiting, the tone of the loop of intestine was increased to 16-28 mm of mercury.

On the 3rd day after irradiation the strength of the intestinal contractions was raised to 30 mm of mercury. The periods of work were lengthened, sometimes to 10 minutes and longer. On the 5th day the same features were observed (Fig. 3).

On the 11th day after irradiation the motor function of the isolated intestine was at the normal level; the 18th day was characterized by a significant depression of the motor activity of the isolated loop of intestine. The maximum strength of the contractions at this time was 6 mm of mercury. The number of contractions fell to 10-11 per minute, and the periods of work were considerable shortened. On the 22nd day the motor function of the isolated intestine rose sharply. The number of intestinal contractions remained normal, but their strength increased to 30 mm of mercury; the tone rose to 14-18 mm of mercury. On the 51st day after irradiation with x-rays the motor activity of the isolated intestine almost reached the normal level. On the 65th day the motor function of the loop of intestine was slightly lowered; the strength of the contractions was 8-14 mm of mercury, and their frequency 9-12 per minute.

Thus, during acute radiation sickness in dogs, caused by irradiation with x-rays in a dose of 400 r, the motor function of an isolated area of the small intestine is quite considerably altered in the direction of depression and also of intensification. It should be pointed out here that under these circumstances there mainly takes place a prolonged increase in the motor activity of the isolated loop of intestine. The motor function of the isolated intestine in different dogs as a rule increases or diminishes at the same time. Thus, for example, between the 3rd and 8th days after irradiation with x-rays the motor activity of the intestine increased in all 3 experimental animals. The same thing could be observed on the 14th-17th, 21st-28th and 31st-48th days. Depression of the motor function in Anakonda and Iula was noted on the 55th-56th day.

The periodicity (phasing) of the secretory processes and absorptive processes of the intestine observed in acute radiation sickness [3-5] are also characteristic of the motor function of the isolated loop of small intestine.

Disturbances of the motor function of the isolated loop of intestine depend evidently on changes in the cholinesterase activity of its tissue. Certain workers have established [6] that under the action of ionizing radiation inactivation of the cholinesterase of the intestine takes place. For this reason the acetylcholine which is continually being liberated is less subjected to destruction, appears in a higher concentration and the motor activity of the intestine which it causes, is increased. Conversely an increase in the cholinesterase activity at certain stages of radiation sickness leads to a fall in the acetylcholine concentration and causes depression of the motor function of the small intestine.

In our experiments the changes in the motor activity of the intestine in normal, unirradiated animals due to proserin and atropine are very reminiscent of the trends of the motor function of the loop of intestine which we observed at various stages of acute radiation sickness. In view of this, we postulate that the disturbances of the motor activity of the intestine in the irradiated dogs may depend on changes in the acetylcholine – cholinesterase system developing in radiation sickness.

SUMMARY

The motor function of the isolated intestinal loop (by Thiry-Vella's method) was studied with the aid of an air-balloon transmission. Experiments were carried out on 3 dogs. Radiation sickness was caused by x-ray irradiation in the dose of 400 r. The results of experiments demonstrated that the motor activity of the isolated intestine is greatly intensified in acute radiation sickness. However, periods of depression and normalization of the motor function were also noted.

LITERATURE CITED

[1] L. G. Voronin, Izv. Nauchn. inst. imeni, Lesgofta, 21, 1-2, 3, 71 (1938).

[2] L. S. Girshberg, Russk. Fiziol. Zhur. 10, 6, 497-511 (1927).

[3] S. R. Perepelkin, Proceedings of the Eighth All-Union Congress of Physiologists, Biochemists and Pharmacologists, pp. 473-474 (Moscow 1955).*

[4] K. V. Smirnov, Proceedings of the Tenth Session of the Institute of Nutrition, pp. 78-79 (Moscow, 1956).*

[5] V. A. Shaternikov, Med. Radio. 4, 61-67 (1956).

[6] J. H. Burn, P. Kordik and R. H. Mole, J. Physiol. 1952, Vol. 116, N. 2, p. 5-6.

[7] R. A. Conard, Am. J. Physiol. 1951, Vol. 165, pp. 375-385.

[8] R. D. Goodman, A. E. Lewis and E. A. Schuck, Ibid. 1952, Vol. 169, pp. 242-247.

* In Russian.

DETECTION OF A STATE OF AUTOSENSITIZATION OF AN IRRADIATED ANIMAL BY MEANS OF LOCAL SKIN TESTS

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We know that allergic reactions play an important part in the development of phenomena which follow the action of ionizing radiation, and the desensitizing perparations are used with success in the treatment of radiation sickness [4]. At present, however, there is very little information about the nature of the allergens which may be responsible for these allergic processes. The possible roles of bacteria, foreign proteins from food and finally, of autosensitization to decomposition products of the body tissues have been suggested.

One of the generally accepted methods of detection of a state of allergy is the intradermal injection of the corresponding allergen to obtain a local hyperemic reaction. So far as we know this method has not yet been used in the study of radiation sickness.

In the present paper we describe the results of an investigation of the reaction of irradiated and healthy animals to the intradermal injection of various allergens.

EXPERIMENTAL METHOD

We used the method of labial injection of allergens, as described by Freund and Stone (into the skin of the upper lip on one side), and recommended by them [7] in experiments on mice and rats when studying the state of active and passive sensitization to foreign protein. Experiments were carried out on 532 white mice and 10 guinea pigs. Identical material was always injected simultaneously into control animals and animals irradiated (on the 1st - 3rd - 5th days) with lethal doses of x-rays (RUM 3 apparatus, 180 kv, 15mA, filter 1 mm copper + 0.5 mm aluminum, distance 50 cm, dose rate 20-21 r/min). The mice were irradiated with a dose of 600 r, causing death of all the animals on the 7th-9th day, and guinea pigs with a dose of 500 r (death on the 8th-10th day). Every day for 7 days the site of injection was examined and some of the animals used for a histological study of the tissues of the upper lip where the injection had been given and also of the symmetrically opposite area of the same animal.

The allergens used were: 1) sterile milk; 2) sterile horse serum; 3) a suspension of a 24-hour agar culture of Escherichia coli with a density of 100 million organisms per 1 ml of physiological saline;* 4) extracts of homologous tissues of healthy and irradiated animals – small intestine, liver, spleen and bone marrow.

The extracts were prepared from freshly removed tissues (of an animal of the same species), cut up finely with scissors and placed in physiological saline to give a concentration of 20%. The supension was filtered through two layers of sterile gauze and injected at once. The volume of material injected varied from 0.05-0.1 ml (usually) to 0.15 ml.

EXPERIMENTAL RESULTS

Injection of the above mentioned substances into healthy animals caused no perceptible external changes

* The injection of large numbers of bacteria is unsuitable, since in consequence of the action of the endotoxin necrosis of the tissue cells takes place, and then in addition to the protein of the bacteria, the products of tissue destruction also become an active factor.

in the skin of the upper lip during inspection for 16-24 hours and later up to 5 days. Histological examination revealed microscopic cellular infiltrations but without necrosis, edema or other changes (Fig. 1). The mice remained alive and active, looked well and had good appetite. The same results were obtained after injection of the animals with these substances 24 hours after irradiation (28 mice, 3 guinea pigs). In spite of the fact that lethal doses of radiation were given, no clinical features of radiation sickness had developed at this period and the animals did not differ in their outward appearance from controls, although a slight loss of weight (of 1-2 g) could be registered, and in a number of cases there were signs of leucopenia.

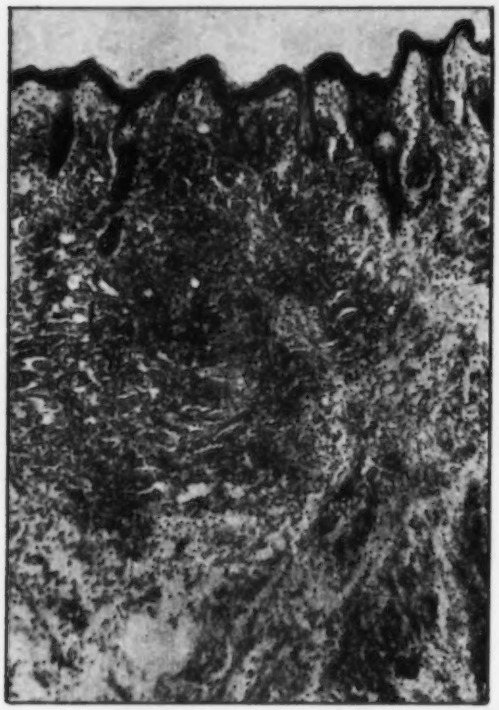

Fig. 1. A microabscess in the skin of the lip of a healthy mouse after injection of an extract of the intestine of an irradiated mouse. Stained with hematoxylin-eosin. Magnification 4 × 10.

Fig. 2. Local hemorrhagic reaction in mice in a positive labial test.

Absolutely different results were obtained after injection of these allergens into mice on the 3rd day, or guinea pigs on the 7th day after irradiation. As can be seen in the Table, it was only in the irradiated animals that a well marked reaction of hyperemic type could be observed after injections of a particular tissue, namely extracts of the small intestine. Under these circumstances acute edema was observed to develop, often involving the whole half of the lip and extending into the neck (Fig. 2). Necrosis of the central part of the edematous tissues very rapidly appeared, sometimes terminating in sloughing of the necrotic tissues. Histological specimens showed total necrosis of the tissues on a background of well marked edema and hemorrhages. No cell reaction on the part of the blood cells (neutrophilic leucocytes) nor in the form of proliferation of the local tissue cells at the periphery of the necrosis could be observed (Fig. 3).

Not only were the hemorrhagic features visible in microscopic preparations, but they could also be clearly seen by external inspection of the animals.

Where the labial reactions were positive, besides the local reaction changes were also observed in the general condition of the animals, which became sluggish, dirty and and unwilling to eat. The body weight fell considerably and the degree of leucopenia increased. Many of the mice and guinea pigs with a strongly positive reaction did not survive for 48 hours after the moment of injection and died on the 4th-5th day after irradiation, although the other irradiated animals from the same batch which were not injected with an extract of homologous intestinal tissue died from radiation sickness only on the 8th-9th day.

We observed the strongest positive reaction in mice after irradiation with a dose of 600 r and injection of extracts of tissue of the small intestine on the 3rd day after irradiation. After the action of larger (750 r) and smaller (400 r) doses this reaction was also observed, but it could be detected only by histological examination. External changes in the form of edema and visible hemorrhages were absent from these anima.. 2 mice). The reaction in mice irradiated with 600 r and injected with tissue extracts after more than 3 days was also less pronounced. Presumably for the most obvious manifestation of this reaction, not only the dose of radiation would be important, but also the presence of specific reactive properties in the

TABLE

Results of Macroscopic Reading of the Labial Reaction in Mice Irradiated with 600 r, after Injection of Allergens on the 3rd Day after Irradiation, and in Healthy Animals

Materials used for labial injection	Mice											
	irradiated						healthy					
	total No. of mice	in which the reaction was					total No. of mice	in which the reaction was				
		–	+	++	+++	++++		–	+	++	+++	++++
Extract of small intestine of healthy mice	34	24	3	5	2	—	25	22	3	—	—	—
Extract of the small intestine of mice irradiated with 600 r (on 3rd day)	58	15	14	5	11	13	28	27	1	—	—	—
Liver, spleen, bone marrow of healthy mice	32	29	3	—	—	—	25	22	3	—	—	—
Liver, spleen, bone marrow of mice irradiated with 600 r (on the 3rd day)	28	27	1	—	—	—	25	24	1	—	—	—
Physiological saline	30	30	—	—	—	—	15	15	—	—	—	—
Suspension of a 24-hour culture of Escherichia coli	28	23	5	—	—	—	20	16	4	—	—	—
Sterile milk	15	13	2	—	—	—	10	10	—	—	—	—
Horse serum	17	14	3	—	—	—	8	8	—	—	—	—
Total . . .	242	175	31	10	13	13	156	144	12	—	—	—

Note: – no reaction; + slight edema; ++ considerable edema; +++ extensive edema and hemorrhage; ++++ edema speading into the neck and head and intensive hemorrhage.

Fig. 3. Section from the site of injection of an extract of the intestine of an irradiated mouse on the 3rd day after the action of x-rays in a dose of 600 r. Necrosis, hemorrhages, absence of infiltration. Stained with hematoxylin-eosin. Magnification 4 × 10.

irradiated animal. Usually we injected 0.1 ml of an extract of intestinal tissue; an increase in this volume to 0.15 ml increased the number of positive reactions.

Thus, of these three different types of allergens which we have mentioned – soluble foreign proteins from mammals, bacterial proteins and homologous tissue extracts – it was only after injection of the last that a local labial reaction, in our opinion hyperergic in character, could be obtained at certain times after irradiation. By the use of this method it is possible to detect in an irradiated animal a state of autosensitization to tissue products of the same species of animal. As can be seen from the results in the Table, a positive labial reaction can be obtained (by this method andiin the conditions of our investigation) only to tissue from the mucosa of the intestine of both healthy and irradiated animals of the same species. Extracts of liver, bone marrow ans spleen failed to give a positive reaction. Tissue from irradiated animals is a more active agent, which may be due, for example, to changes in the antigenic properties of the tissues in an irradiated animal [5]. The fact that intestinal extract shows the greatest activity has a definite relation to its high degree of trauma after exposure to radiation [1, 2, 3, 6, 8].

It is interesting that the injection of living bacteria in the doses given not once caused such a reaction, although cultures taken from the site of injection demonstrated the presence of proliferation of the organisms in the tissue of the lip. These findings are in conformity with the indications of a lowering of the reactivity of the irradiated animal towards bacteria and shed doubt on a leading role for the latter as a source of sensitization in radiation sickness. It may be assumed that in association with the lowered reaction of the irradiated animal, the proliferation and abundant accumulation of bacteria are not the cause of radiation sickness but its consequence, complicating the later course of the disease.

In all the variants of the experiments we injected into the skin of the upper lip of the animals,as extracts, ready prepared products of destruction of cells from different organs, and as seen from the results given the activity of the extracts in the test studied was not identical. This could depend both on variations in the content of allergens in the different organs, and on their partial or total destruction in the process of preparation of the extracts. Finally, all the allergens might not pass into the extracting fluid from the cells and be active in the dose which we used.

We attempted to discover the role of the products of cell destruction formed in the tissue of the lip itself under the influence of indifferent cell-destroying agents. For this purpose we injected into the skin of the lip of animals 0.1 ml of distilled water (49 mice). A few hours later edema developed at the site of injection in both healthy and irradiated animals, and disappeared after 24 hours. In healthy mice, and in mice taken within the first 24 hours after irradiation with a dose of 600 r, no perceptible changes appeared subsequently at the site of injection. In histological preparations the presence of only slight cellular infiltration could be observed. If the distilled water was injected on the 3rd day or later, then the mice irradiated with 600 r developed a typical positive labial reaction, with edema, tissue necrosis and hemorrhages. However, it appeared somewhat later than after injection of intestinal extracts (not 18-24 hours, but 3-4 days after the test injection).

Thus, by the labial injection of extracts of certain homolgous tissues and by the creation at the site of injection of conditions in which products of their destruction are formed and partially extracted in the tissues themselves, a local reaction of a hyperergic type can be produced in an autosensitized animal.

The study of these local manifestations of the general state of autoallergy is of theoretical and practical importance not only in radiation sickness but also in other diseases associated with the action of products of tissue destruction on the body. It is essential to point out the importance of injecting the allergens into definite areas of the body, most sensitive to allergic reactions, for example the skin of the upper lip, since we have satisfied ourselves that negative results are obtained if these tests are done on the skin of the thigh or the abdomen.

SUMMARY

The authors studied the possiblity of detecting the state of sensitization of the irradiated body to the products of disintegration of its own tissues and of those obtained from animals belonging to the same species (labial administration by Freund-Stone's method). It was established that in definite doses of irradiation a hyperergic reaction with edema, necrosis and hemorrhages occurs at the site of administration at a definite period of the radiation sickness. Such a reaction is caused only by the extracts of homologous tissues and not by the introduction of bacteria or foreign proteins. The reaction is negative in the nonirradiated animals and during the first 48 hours after the irradiation.

LITERATURE CITED

[1] The Biological Action of Radiation and the Clinical Features of Radiation Sickness, Ed. by A. Ignat'ev, Moscow, (1954).*

[2] N. N. Klemparskaia, Biull. Eksptl. Biol. i Med. 5, 22 (1956).**

[3] N. A. Kraevskii, Essays in the Pathological Anatomy of Radiation Sickness, Moscow (1957).*

[4] N. A. Kurshakov, Klin. Med. 33, 6, 12 (1956).

[5] R. V. Petrov, and L. I. Il'ina, Biull. Eksptl. Biol. i Med. 4, 59 (1956). **

[6] R. Conard, Radiat. Research. 1956, Vol. 5, N. 2, p 167.

[7] J. Freund and I. Stone, Journ. of Immunol. 1956, Vol. 72, No. 2, p. 138.

[8] H. Quastler, Radiat. Research 1956, Vol. 4, N. 4, p. 303.

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* In Russian.

** Original Russian pagination. See C. B. Translation.

THE INFLUENCE OF THE EXTRACARDIAC NERVOUS SYSTEM ON THE FUNCTIONAL STATE OF THE HEART IN EXPERIMENTAL MYOCARDIAL INFARCTION

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Some workers consider the leading factor in the etiology of myocardial infarction to be a functional disturbance of the coronary circulation, others – atheromatous changes in the vessels of the heart. In recent years these factors have come to be regarded as complementary to each other, and in addition the factor of the functional load has aroused increasing interest.

In 1953, for instance, in acute experiments on dogs with experimentally induced myocardial infarction, we showed that if the contractions of the heart were greatly intensified, myocardial infarction developed significantly more quickly and was more extensive than after ligation of the descending branch of the left coronary artery alone. Intensification of the contractions of the heart was produced by prolonged stimulation of the peripheral section of the vagus nerve with an electric current or by increasing the tonus of the vagus nerve center by the intravenous injection of adrenalin on a background of the action of morphine [6, 7, 8, 9].

From these experiments it can be seen that the discrepancy arising between the strength of the cardiac contractions and the coronary blood supply during coronary insufficiency may lead to the development of a myocardial infarct or may considerably aggravate the course of an already existing infarct.

Our findings were subsequently confirmed in work by A. I. Strukov and S. A. Vinogradov [2, 10].

The hypothesis of the discrepancy between the strength of the contractions of the heart and the blood supply to the myocardium as the cause of production of a myocardial infarct in man was first put forward by F. A. Andreev [1]. Recently clinicians have attached ever increasing importance to the functional load in the development of a myocardial infarct not only in coronary insufficiency but also in healthy young persons during excessive physical effort [11].

In the study of the pathogenesis of the myocardial infarct in our laboratory, as in the case of other workers, it was observed that in the process of development of the infarct itself and in its outcome, the state of the collateral coronary circulation and of the intima of the coronary vessels has great significances. Where there is a well developed collateral system ligation of the descending branch of the left coronary artery in dogs does not lead to extensive infarction [5].

It was shown by experiments of long duration on dogs in our laboratory that after ligation of the descending branch of the left coronary artery, severe polytopic ventricular extrasystole, which appear without compensatory pauses, arise during the first few days after operation. As the researches of A. I. Smirnov, A. I. Shumilina and O. V. Ul'ianov showed, extrasystole is closely connected with the influence of the vagus nerve on the heart [7]. This provided us with a basis for a more detailed study of the functional condition of the tonus of the vagus nerve center and of its influence on the heart during experimental myocardial infarction.

TABLE

Changes in the Cardiac Activity During Experimental Myocaridal Infarction

Name of dog	Date of observation	Control experiment		Operation		Day after operation																	
						2nd		3rd		4th		5th		6th		7th		8th		9th			
	Indices of cardiac activity / Experimental conditions	13/VI 1957		17/VI 1957		18/VI 1957		19/VI 1957		20/VI 1957		21/VI 1957 r.		22/VI 1957				24/VI 1957					
		pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles	pulse rate	presence of extra-systoles		
Usach	Initial findings	120	Absent	—	—	120—140	Present	130—140	Present	130—140	Absent	110	Absent	100	Absent	—	—	96	Absent	—	—		
	After injection of morphine	56	»	—	—	—	—	130—140	Respiratory arrhythmia	130—140	Present	90	Present	66	»	—	—	78	»	—	—		
	After injection of $CaCl_2$	34	»	—	—	—	—	130—140	The same	140—150	»	74	»	56	»	—	—	50	»	—	—		
	Date of observation	4/IV 1957		8/IV 1957		9/IV 1957		10/IV 1957		11/IV 1957		12/IV 1957		13/IV 1957						16/IV 1957	
Dzhul'-bars	Initial findings	90	Absent	110	Absent	120—130	Present	112	Present	100	Present	110	Absent	96	Absent	—	—	—	—	110	Absent		
	After injection of morphine	50	»	44	»	—	—	130	»	88	»	76	Present	54	»	—	—	—	—	90	»		
	After injection of $CaCl_2$	36	»	—	—	—	—	140	»	74	»	68	»	42	»	—	—	—	—	68	»		
	Date of observation	28/II 1957		5/III 1957				7/III 1957		8/III 1957		9/III 1957				11/III 1957		12/III 1957			
Sultan	Initial findings	90	Absent	—	—	—	—	140	Present	130	Present	140	Present	—	—	130	Absent	100	Absent	—	—		
	After injection of morphine	70	»	—	—	—	—	140	»	130	»	120	»	—	—	110	»	90	»	—	—		
	After injection of $CaCl_2$	50	»	—	—	—	—	140	»	130	»	100	»	—		60	»	72		—	—		

The tonus of the vagus nerve center may be increased by injection of morphine. It is intensified still more if, in addition to the effect of morphine, calcium chloride or adrenalin [3, 4] are injected intravenously. We made use of these findings in our experiments.

EXPERIMENTAL METHOD

The experiments were carried out on 15 adult dogs, on which the operation of ligation of the descending branch of the left coronary artery was performed.

The operations were carried out under sterile conditions, under morphine-urethane or morphine-evipan anesthesia. After preparation of the field of operation with iodine solution an incision 10 cm long was made between the fourth and fifth left ribs. As a preliminary measure the dog was changed over to artificial respiration. After the pleural cavity had been opened, the ribs were displaced with retractors, the pericardium opened and ligation of the descending branch of the left coronary artery performed. The pericardium was then partially excised and the wound closed in layers. In order to prevent postoperative complications penicillin was injected intramuscularly during the first few days after the operation.

The development of an infarct of the myocardium as a result of this procedure was confirmed electrocardiographically, and later by morphological examination.

When 24 hours had elapsed after the operation, an increase in the tonus of the vagus nerve center was produced in each experimental dog every day for 1-1½ weeks by the injection of morphine and calcium chloride. Morphine was injected subcutaneously in a dose of 1-5 ml of a 1% solution, depending on the weight of the animal. Calcium chloride was injected intravenoulsy in a dose of 0.05 g per 1 kg body weight of the animal.

In control experiments on the same dogs before operation, an increase in the tonus of the vagus nerve center was produced in an identical manner.

In certain cases acute experiments were performed on the 3rd-4th day after operation in which, under morphine-urethane or morphine-evipan anesthesia, both vagus nerves were divided and their peripheral ends then stimulated with an electric current. Morphine was injected in a dose of 1 ml of a 1% solution per 1 kg body weight of the animal, and evipan was injected intraperitoneally or intravenously in the form of a 10% solution before the onset of anesthesia.

In individual experiments a 0.25% solution of novocain was injected into the trunk of the vagus nerve and atropine was injected subcutaneously in a dose of 1-2 ml of a 1 : 1000 solution.

In all the experiments the electrocardiogram was recorded, the rate of the cardiac contractions was measured, and during the actute experiments the arterial pressure was registered with a mercury manometer. Stimulation of the peripheral end of the vagus nerve was carried out by means of a Dubois-Raymond induction apparatus.

Altogether 10 control, 12 acute and 47 chronic experiments were performed.

EXPERIMENTAL RESULTS

The changes obtained by the influence of tonic impulses from the vagus nerve center during experimental myocardial infarction in dogs are illustrated in the Table and by kymograms and electrocardiograms of typical examples.

The investigations carried out showed that in all the control experiments the intravenous injection of calcium chloride, when superimposed on the effect of morphine, or prolonged stimulation of the peripheral portion of the vagus nerve with an electric current leads to a significant slowing of the rate and strengthening of the contractions of the ventricles of the heart (see Table and Fig. 1).

On the development of experimental myocardial infarction in the dogs, after 18-24 hours there appear polytopic ventricular extrasystoles of varying intensity, without compensatory pauses, and these gradually disappear on the 5th-6th day after operation (see Table and Fig. 2).

As already shown in our laboratory, extrasystoles appear only in case of development of experimental myocardial infarction. If the ligation of the descending branch of the left coronary artery did not result in the formation of a myocardial infarct, extrasytoles were absent.

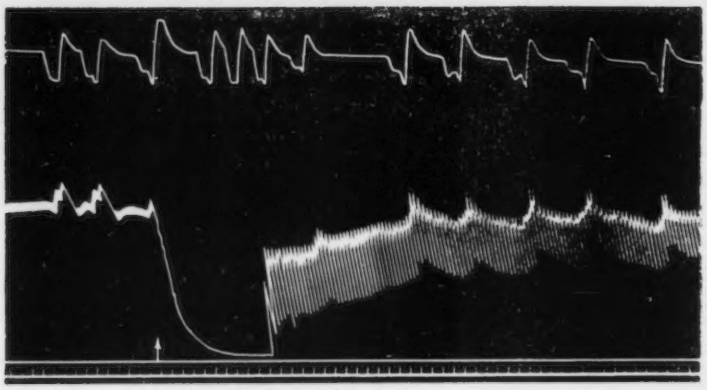

Fig. 1. Stimulation of the peripheral portion of the vagus nerve in a dog under morphine-evipan anesthesia before ligation of the descending branch of the left coronary artery. Significance of the curves (from above downwards): respiration, arterial pressure in the femoral artery, stimulation marker, time marker, which is also the zero line.

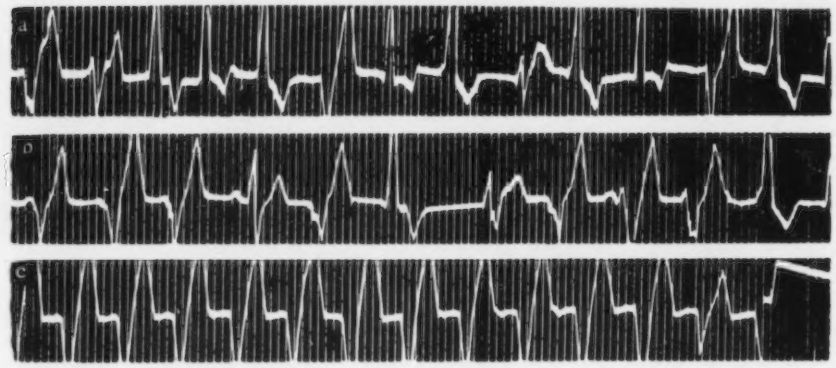

Fig. 2. Extrasystoles in the dog Sultan, lead II, experiment dated March 7, 1957. a) Polytopic ventricular extrasystoles on the 2nd day after ligation of the descending branch of the left coronary artery; b) electrocardiogram 10 minutes after intravenous injection of calcium chloride superimposed on the effect of morphine. The number of extrasystoles of the left ventricle is increased; c) electrocardiogram 30 minutes after the injection of calcium chloride superimposed on the effect of morphine.

After operation, increase of the tonus of the vagus nerve center by the intravenous injection of calcium chloride, superimposed on the effect of morphia, led to intensification of the existing extrasystoles for the first 5-6 days, without any slowing or strengthening action on the heart.

After the injection of calcium chloride the polytopic extrasystoles gradually changed into extrasystoles of the left ventricle (see Fig. 2).

After division of both vagus nerves, in more than 50% of cases the extrasystoles disappeared. In those cases where they continued, a considerable reduction in their number was observed. After vagotomy, stimulation of the peripheral portion of the vagus nerve with an electric current always led to the appearance of extrasystoles or to the sharp intensification of existing extrasystoles of the same type as before the vagotomy, and under these circumstances they lasted for the whole period of action of the electric current on the nerve [6] (Fig. 3). If after

division of both vagus nerves the extrasystoles disappeared, stimulation of the ansa of Vieussens did not cause them to appear, whereas in the same experiment subsequent stimulation of the peripheral portion of the vagus nerve led to reappearance of the extrsystoles. This was shown by O. V. Ul'ianova in our laboratory.

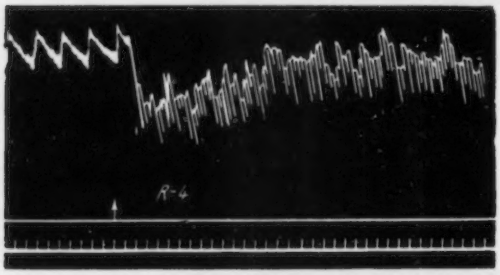

Fig. 3. Stimulation of the peripheral portion of the vagus nerve in a vagotomized dog on the third day after ligation of the descending branch of the left coronary artery. Polytopic ventricular extrasystoles.
Significance of the curves (from above downwards): arterial pressure in the femoral artery, stimulation marker, time marker, which is also the zero line.

The extrasystoles were intensified during mechanical stimulation of the vagus nerve by means of injection of 2-3 ml of physiological saline into its trunk, and they disappeared after injection in a similar manner of 3-4 ml of a 0.25% solution of novocain.

Subcutaneous injection of 1-2 ml of a 0.1% solution of atropine did not abolish the extrasystoles.

Restoration of the usual influence of the vagus nerve, in the form of slowing of the rate and strengthening of the contractions of the heart, was noticeable on the 5th-6th day (see Table). The slowing down of the rhythm and strengthening of the contractions of the heart took place both after intravenous injection of calcium chloride in association with the action of small doses of morphine, and during prolonged stimulation of the peripheral section of the vagus nerve with an electric current.

In none of our experiments did the extrasystoles, intensified by raising the tonus of the vagus nerve center or by stimulation of the peripheral section of the vagus nerve, lead to the development of ventricular fibrillation in the heart.

As already shown by research in our laboratory, the appearance of extrasystoles is characteristic of experimental myocardial infarction. In the development of such extrasystoles an important part is played by impulses coming from the vagus nerve center. This is also confirmed by our present investigation. The findings mentioned above show that during the first few days after the development of experimental myocardial infarction, the influence of the vagus nerve on the heart is shown not by slowing of the rate and strengthening of the contractions of the heart but by the appearance of ventricular extrasystoles, which may be in consequence of the change in the functional condition of the heart during infarction.

After 5-6 days, when the initial organization of the myocardial infarct takes place, the functional stage of the heart gradually returns to its original, and concurrently with the gradual disappearance of the extrasystoles the usual action of the vagus nerve is restored, in the form of slowing of the rhythm and strengthening of the contractions of the heart.

It may be mentioned that the extrasystoles which we observed, which were mainly due to tonic impulses from the vagus nerve center, did not significantly complicate the activity of the heart, since they rapidly passed off (on the 5th-6th day) and in our experiments on dogs they never led to ventricular fibrillation.

The results obtained indicate yet again that in the study of the pathogenesis of myocardial infarction it is essential to take into consideration both the functional condition of the heart and also the influence exerted on it by the extracardiac nervous system.

SUMMARY

Experiments were performed on 15 dogs in acute and chronic experiments. Myocardial infarction was induced by the ligature of the descending branch of the left coronary artery. To raise the tone of the vagus intravenous injections of calcium chloride were given on the background of morphine action before and after the operation. ECG readings were taken, the blood pressure measured and the number of cardiac contractions and respirations recorded.

Experiments demonstrated that increased tone of the vagus nerve center during the first 4-5 days after the

induction of the experimental myocardial infarction does not result in the decrease of the rhythm of intensification of the cardiac contractions. However, it intensifies the extra systoles of the cardiac ventricles. The inhibiting effect of the vagus nerve on the heart is reestabished on the 4th-25th day after the operation which coincides with the disappearance of the extrasystole.

The above-mentioned changes in the action of the vagus nerve on the heart depend to a great extent on the changes in the functional condition of the heart as a result of myocardial infarction. An explanation of the genesis of extrasystoles in human myocardial infarction may be sought in these data.

LITERATURE CITED

[1] F. A. Andreev, The Nervous Regulation of the Circulation and Respiration, pp. 107-113, Moscow (1952).*

[2] S. A. Vinogradov, Biull. Eksptl. Biol. i Med., 41, 5, 27-32 (1956).**

[3] G. G. Eliseev, Kubanskii Nauchno-Med. Vestnik, 5-8, 213-220 (1921).

[4] M. S. Keropiian and E. I. Turbina, Ibid, 4, 47-63 (1924).

[5] A. V. Kuz'mina-Prigradova, Biull. Eksptl. Biol. i Med. 42, 9, 67-71 (1956).**

[6] A. I. Smirnov and A. I. Shumilina, Klin. Med. 2, 62-77 (1955).

[7] A. I. Smirnov, A. I. Shumilina and O. V. Ul'ianov, Proceedings of the Eighth All-Union Contress of Physiologists, Biochemists and Pharmacologists, pp. 562-564, Moscow (1955).*

[8] A. V. Smol'iannikov and A. K. Apatenko, Transactions of the All-Union Conference of Pathological Anatomists, pp. 258-263, Moscow (1956).*

[9] A. V. Smol'iannikov, Arkh. Patol. 5, 3-13 (1957).

[10] A. I. Strukov and S. A. Vinogradov, Proceedings of the 14th All-Union Congress of Physicians, pp. 139-141, Moscow (1956).*

[11] S. Bloch and S. Tara, Arch. mal. profess. 1952, v. 13, p. 278-279.

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* In Russain.

** Original Russian pagination. See C. B. Translation.

ELECTROCARDIOGRAPHIC EXAMINATION OF THE HEART IN RATS AFTER INJURY TO THE MYOCARDIUM

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Regeneration of the myocardium in rats was shown to be possible in our collective work [2]. We studied this process in a combined manner, using the methods of experimental morphology, biochemistry, physiology and histology.

The aim of the present work was to trace the progress of the changes in the electrocardiogram during healing of the myocardium after injury and also during stimulation of the process of regeneration with biological preparations.

The electrocardiogram of normal rats has been studied by many workers [4-6, 7, 8]. It differs from the electrocardiogram of other animals by the definite stability of its main waves. It is not possible to take an electrocardiogram from an unanesthetized rat on account of muscle tremor in the rats which are restless when placed in an outstretched position, and furthermore the type of anesthesia (urethane, ether etc.) also affects the character of the electrocardiogram.

In the experiment we used 100 adult (male) white rats, weighing from 250 to 400 g. Electrocardiograms of 39 of these were studied in experiments with injury. The electrocardiograms were taken under urethane anethesia (3-5 ml of a 5% solution given intraperitoneally). The rat was secured to the table by its paws with soft tape, in the prone position. The electrodes used were fine hypodermic needles, inserted under the skin of the limbs. Electrocardiograms were taken with a type EKP 4 electrocardiograph (EMA factory). Film was fed at the rate of 80 mm/sec. Electrocardiograms were taken with leads I, II and III, but since with lead I the R wave was extremely low and all the other waves were completely absent, lead II and III elecrocardiograms were selected for study (Fig. 1). (in this and in all succeeding experiments the curves shown are from lead II).

The rate of the undamaged heart in the adult anesthetized rat varied from 360 to 480 beats per minute. The amplitude of the waves in the normal electrocardiogram of the rat is considerable less than in man. In the normal rat the P-Q interval from the beginning of the P wave to the beginning of the Q wave, corresponding to the atrioventricular conductivity, is 0.050-0.065 seconds. No realtionship was found between the length of this interval and the rate of the heart. The QRS complex – the time for the spread of excitation through the myocardium of the venticles – in the same conditions was 0.018-0.020 seconds. Here also no relationship was established between the duration and form of the QRS complex and the rate of the heart. The QT segment – the ventricular complex of the electrocardiogram – varied in different animals between limits of 0.063 and 0.110 seconds.

The length of this particular interval depends on the rate of the heart just as is observed in the human electrocardiogram. The amplitude of the waves was characteristically as follows: the P wave was positive in all cases in leads II and III, and the variational deviations of the P wave were within normal limits; the R wave also was always positive and its height in lead II was 0.14-0.36 mv and in lead III – 0.10-0.25 mv; the height of the T wave varied from a smoothed out plateau to a well marked positive crest; the S wave in lead III was present in 37.5% of cases, but in lead II in only 10.2%. The Q wave was not found at all.

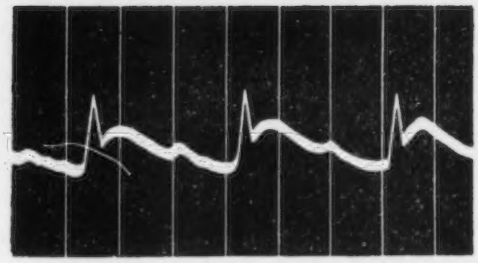

Fig. 1. Electrocardiogram of the healthy rat.

The rats selected for the experiment mainly had a normal type of electrocardiogram. As a result 33 of the 39 showed a normal type, three showed signs of left and three of right axis deviation. The type of electrocardiogram was determined by the height of the main waves in leads II and III. The normal electrocardiogram was studied in rats which had been under our observation for a long time. The electrocardiogram was normally taken in each animal 2 or 3 times in the 2 weeks before operation.

The operation was carried out as follows. The animal was anesthetized with urethane, the fur removed from the operation field, and the heart exposed with sterile precautions in the fourth intercostal space. An artificial respiration apparatus was used. Air was passed into the trachea through a cannula. The beating heart was momentarily damaged with a diathermocoagulation; a whitish spot with a diameter of 4 mm on the average appeared on its surface. The apex of the cone of necrotic tissue, similar to that seen in an infarct, was at a depth of 3-5 mm in the myocardium. The wound was sutured in layers, and an electrocardiogram taken immediately afterwards. Subsequently electrocardiograms were taken 1, 3, 6, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120 and 160 days after the operation.

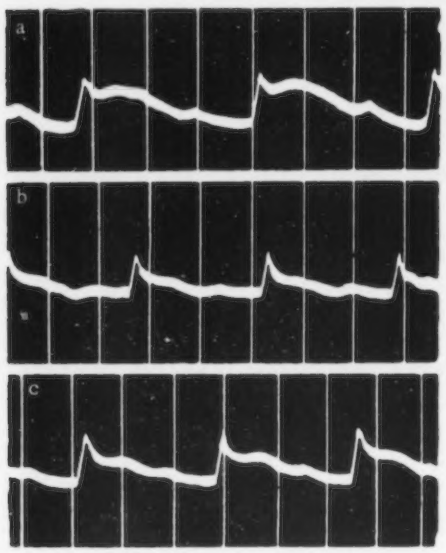

Fig. 2. Electrocardiogram of a rat at different intervals after injury to the heart. a) Acute stage; b) subacute stage; c) stage of scar formation.

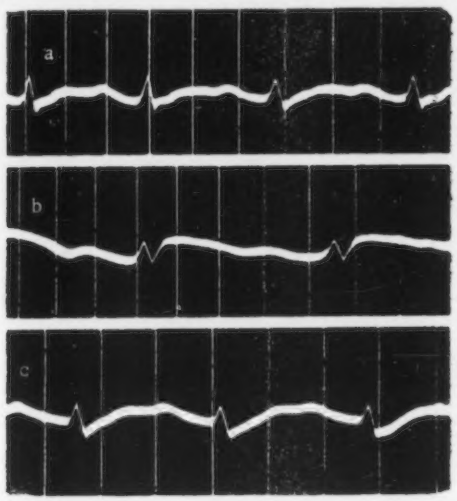

Fig. 3. Electrocardiogram of a rat during experimental injury to the heart and the subsequent injection of a hydrolyzate of cardiac muscle. a) Normal b) acute stage; c) stage of scar formation.

In a control series in which the heart was damaged without any additional treatment, on the basis of the characteristic changes in the electrocardiogram 3 stages of injury were established. In experiments on rabbits [1] in which the coronary arteries were ligated, characteristic changes in the electrocardiogram were also found at certain times after operation.

In our experiments the acute stage (Fig. 2, a) begins immediately after operation. In this period the following characteristic signs are observed on the electrocardiogram: the RS-T interval is markedly displaced upwards, forming a monophasic curve; the T wave is "gigantic" and positive; the S wave if present normally is deepend; the rate is considerably slowed: 310-340 beats per minute; the atrioventricular conduction is retarded and PQ

measures 0.062-0.080 seconds. The electrical systole of the ventricles of the heart is lengthened and QT measures 0.100-0.137 seconds. The changes continue for several hours, and in some animals for 2-3 days after the operation. During this period the animals are very weak and a large proportion of them die in the course of it. In the weakest rats the heart rate falls to 180 per minute. After only 24 hours, in the majority of animals the character of the electrocardiogram is sharply altered, changing over from the acute to the subacute stage (see Fig. 2, b). The T wave, instead of being "gigantic," is smoothed out an may even become slightly negative. The RS-T interval is left in isolation and the rhythm becomes slightly quicker than normal: 420-500 beats per minute. The PQ interval measures 0.050-0.062 seconds and QT 0.062-0.081 seconds. The amplitude of R is increased; R_{II} measures 0.17-0.14 mv, R_{III} is 0.17-0.50 mv. The subacute stage lasts for 4 to 8 days. With reestablishment of RS-T on its original line, the longest stage begins – the stage of scar formation, which lasts for different lengths of time in different animals. The rate of the heart attains the normal value; the length of the PQ and QT intervals hardly differs from normal. The amplitude of the R wave is reduced. At later periods of the stage of scar formation the electrocardiogram may approach closer to normal. In two of the seven cases in the control series the electrocardiogram returned to normal in one animal after 30 days and in the other 40 days after operation. However, after 50 days and continuing until the 160th day, their electrocariogram once more returned to that of the stage of scar formation.

In the stage of scar formation (see Fig. 2, c) the electrocardiogram differs from normal by the shape of the T wave, which merges with the P wave at a height above the base line. The PQ interval is displaced upwards at an angle to the base line. The R wave is diminished in height, and its apex is blunter than normal.

In the next series of experiments an extract of hydrolysate of cardiac muscle was injected into the animals immediately after operation. The biological preparations were injected 10 times on alternate days [2]. Electrocardiograms were taken under normal conditions and at the same times as in the control series. In this series only two stages could be identified – an acute stage and a stage of scar formation. The subacute stage was probably very much shortened. The duration of the acute stage was different in the different animals. They could be separated into two groups. In the animals in which the acute stage lasted several hours, after only 24 hours the characteristic curve of the stage of scar formation was registered. On subsequent days the stage of scar formation again reverts to the acute stage; finally, after 7 days the stage of scar formation becomes stabilized. The return of the electrocardiogram to normal was not observed in these animals. In the rats of the other group the length of the acute stage (see Fig. 3, b) was increased to 2-3 days. The next stage was that of scar formation and the character of the curve was close to normal. Only 11 days after operation, in 50 per cent of cases the electrocardiogram returned to normal and remained normal until the 30th day after operation (see Fig. 3, c). The characteristics of this stage were the same as in the previous series of experiments. Comparison of the electrocardiograms of the rats in these 2 series of experiments will show that in the series of animals treated with hydrolysate the process of wound healing takes place more rapidly than in the control series. Furthermore, in 50% of these cases, return of the electrocardiogram to normal was observed.

In a third series of experiments the animals were injected with extracts of cardiac muscle [2]. No essential differences in the electrocardiogram in comparison with the controls could be observed. In some animals, 3 days after operation the subacute stage once more returned to acute, and this lasted until 5 days after operation. Later the stage of scar formation appeared and continued until the 160th day after operation without any return to normal.

If the electrocardiographic findings are compared with the histological picture of the experimental hearts, a certain parallel may be observed between the functional and morphological changes in the heart. The histological findings show that [2] in the control series, 20 days after operation, in the region of the injury, foci of regenerating muscle are seen which have become resolved 46 days after the operation, and become converted into a connective tissue scar. We see a similar picture in the electrocardiogram: in some animals 30-40 days after operation the electrocardiogram has become normal, and after 50 days it once more changes into the stage of scar formation. In the experiments with hydrolysate a return of the electrocardiogram to normal from the stage of scar formation is also observed, but this takes place much earlier and is more stable than in the control series. In this series of experiments, only 11 days after operation in 50% of cases a normal electrocardiogram is registered. Histological examination shows that at this same time, on the 10th-13th day after operation, regeneration of muscle fibers is taking place in the focus of injury.

SUMMARY

Three stages of myocardial healing were established in injury of the heart wall in rats (by means of electrodiathermocoagulator) and the following development of an infarct-like focus, i.e. acute, subacute and the stage of of scar formation. Changes in the electrocardiogram correspond to these stages.

In experiments with additional administration of biopreparations stimulating the regeneration after the heart injury the best effect was obtained by using the hydrolysate of the heat muscle. Thus, after the 10-fold administration of this hydrolysate a shortening of the subacute stage and the earlier appearance of the stage of scar formation was noted.

In this series of experiments a stable return of the electrocardiogram to the normal was noted 11 days after the the operation in 50% of the cases.

In comparing the physiological data with the histological one it was established that the normalization of the electrocardiogram corresponds to the time of regeneration of muscles at the site of the injury.

LITERATURE CITED

[1] Kh. Ia. Gorodins'ka, Fiziologichn. Zhur. 2, 1, 53-57 (1956).

[2] L. V. Polezhaev, L. V. Akhabadze, N. A. Zakharova and V. L. Mant'eva, Doklady Akad. Nauk. SSSR, 119, 5 (1958).•

[3] I. A. Sapov, Biull. Eksptl. Biol. i Med. 40, 9, 26-30 (1955).

[4] J. Boros and B. Boros, Cardiologia, 1948, Bd. 13, S 325-251.

[5] J. Buchanan, J. Physiol. 1909, Vol. 72, N. 39.

[6] P. Eggers and H. Schumann, 1940, Bd. 137, S. 710-717.

[7] E. Heise and K. H. Kimbel, Ztschr. Kreislaufforsch. 1955, Bd. 44, S 212-221.

[8] E. Lepeschkin, Das Electrokardiogramm, Dresden-Leipzig, 1947.

[9] P. Lukoschek and J. Thiesen, Ztschr. Kreislaufforsch. 1954, Bd. 43, S. 115-120.

[10] W. Massmann and H. Opitz, Cardiologia, 1954, Bd. 24, N. 1, S. 54-64.

[11] W. Massmann and H. Opitz, Ztschr. ges. exper. Med. 1954, Bd. 124, S. 35-43.

•See English translation.

PROTECTIVE AND ADAPTATIONAL REACTIONS OF THE ANIMAL BODY TO THE INJECTION OF SMALL DOSES OF FOREIGN BLOOD

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The study of the protective reactions of the body in response to the action of pathogenic stimuli is of great importance. In the present paper we describe the results of an investigation of the protective and adaptational reactions arising after injection of small doses of foreign blood into animals (white rats).

EXPERIMENTAL METHOD

The investigation was carried out on the model which we have devised to reproduce heterotransfusional shock accompanied by acture pulmonary edema. In numerous experiments a variety of substances and agents (adrenalin, calcium chloride, potassium chloride, calcium chloride with glucose or adrenalin, quinine, atropine, novocain, various narcotics, novocain block of the vagus and sympathetic trunks in the neck, division of the vagus nerves in the neck or below the diaphragm, section of the spinal cord between the first and second thoracic vertebrae and so on) has been tested without showing any marked effect on the course of the shock. The only factor found to be effective was the preliminary intravenous injection of ox blood (plasma or serum) in doses which did not produce signs of shock. The mechanism of this phenomenon was the subject of our investigation.

EXPERIMENTAL RESULTS

If a few minutes before the infusion of a lethal dose of ox blood (plasma or serum) into a rat, a small dose of the same blood (plasma or serum), causing no perceptible disturbances of function of the animal, is injected intravenously into the rat, the subsequent injection of the foreign blood is tolerated by the animals in the majority of cases without any signs of shock; only a well-marked hemoglobinuria is observed.

A similar phenomenon after the injection of incompatible blood or serum was observed in dogs [10, 11 and others], cats [11], rabbits [3, 13], and also in man [2, 6, 12, 16 and others]. A rapid protective reorganization was also observed during the action on animals of microorganisms and their toxins [1, 7, 8 and others], tissue extracts [13, 14] and also of certain other protein and nonprotein substances. These phenomena have been described under various names ("tachyphylaxis", "allergization," "desensitization" and so on). We shall call the phenomenon which we observed tachyphylaxis [4, 7].

The phenomenon of tachyphylaxis appeared in our experiments after a preliminary injection of ox blood in doses of 0.5 ml/kg and over. Usually we employed doses of 2 to 3-5 ml/kg. Without causing signs of shock, as a rule these doses were sufficient to preserve the rats from shock after the subsequent injection of shock-producing doses of ox blood (Figs., 1 and 2).

Known shock-producing doses of foregin blood were injected into rats from 5 minutes - 1 hour after the injection of protective doses in 86 experiments. In 44 animals shock did not develop, 9 animals died from shock with acture pulomary edema, and in the remaining experiments only slight manifestations of shock were observed.

Of the 9 experiments which terminated fatally, in four the ox blood possessed very high shock-producing powers, and the severity of the shock was not reduced by two or three preliminary injections of small doses of the same blood. In a number of experiments it was demonstrated that the absence of tachyphylaxis depended on the individual reactive peculiarities of the rats.

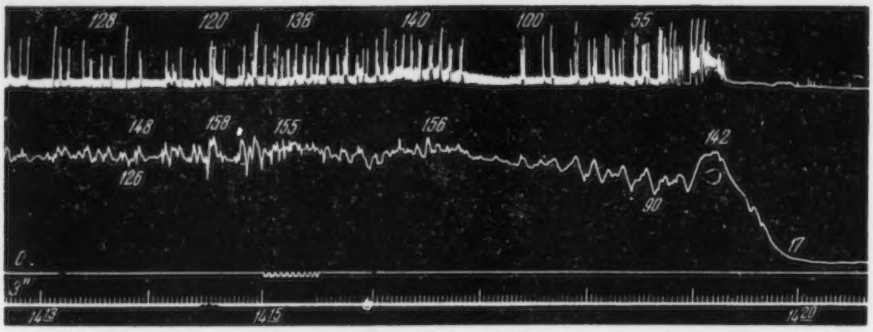

Fig. 1. Changes in the respiration and arterial pressure after injection of a lethal dose of ox blood into a white rat. Significance of the curves (from above downwards): respiratory movements (the figures indicate the number of respirations in one minute); arterial pressure (the figures indicte the height of the arterial pressure in mm of mercury); zero line and stimulation marker – injection of ox blood in a dose of 15 ml/kg; time marker (3 seconds). Weight index of the lungs, determined after the death of the rat, 18.6.

We may note specially that in 2 experiments in which the arterial pressure and respiration were registered, after the injection of the "prepared" rats with a lethal dose of ox blood, not only did the arterial pressure not fall after the usual initial phase of elevation, but it considerably increased (to 200 and 210 mm of mercury). In some experiments, besides registration of the arterial pressure and respiration, a record was made on the drum of the kymograph of the volume of the kidney, by means of an oncometer. The experiments showed that the preliminary injection of small doses of blood protects the renal vessels from the constriction observed during shock.

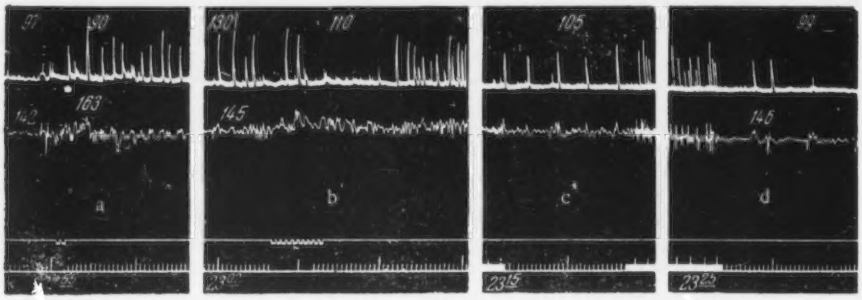

Fig. 2. Changes in the respiration and arterial pressure after injection of a white rat with a lethal dose of ox blood (15 ml/kg) after the preliminary injection of a small dose (2 ml/kg) of the same blood. Significance of the curves the same as in Fig. 1. Weight index of the lungs, determined after death of the animal, 6.1.

Thirty rats in which fatal shock did not develop were killed in order to determine the weight index of the lungs. On the average this amounted to 7.7, varying between 4.9 and 9.5, i. e. it was considerably lower than that observed in animals dying from shock (from 10.2 to 38.7). Histological investigations showed that the appearance of a certain degree of edema was often found in lungs which hardly differed from normal in their weight and external appearance.

In order to discover the duration of action of the protective and adaptational reorganization which we were studying, in 38 experiments white rats were injected with a lethal dose of ox blood from 2-7½ or from 50 minutes to 6 hours 55 minutes after injection of the small dose. Only 3 minutes after injection of the small dose of ox blood it was possible to observe the protective action, preventing death of the animals after the subsequent injection of blood. This action continues for 1½ hours. Some of our experiments on rabbits and dogs, and also clinical observations, when compared with the experiments and clinical observations of other authors [2, 3, 4, 6, 10, 11, 12 and others], show significant species differences in the ability of the animal to reproduce tachyphylaxis during infusions of foreign blood.

We also attempted to study the specificity of tachyphylaxis. Workers who have dealt with this problem [11, 14, 15 and others] are inclined to deny its existence. In 2 experiments, in one of which a record was made of the arterial pressure and respiration, tachyphylaxis was induced by human blood in a dose of 5 ml/kg given 10 minutes before the injection of ox blood. Tachyphylaxis did not develop. In 10 experiments tachyphylaxis was induced by dogs' blood in a dose of 1.35-15 ml/kg, given 10-16 minutes before the injection of ox blood. In 6 experiments the shock reaction was clearly diminished and in four tachyphylaxis was not observed. No direct connection could be observed between the size of the dose of the first injection and its effectiveness in these experiments. In 11 experiments, in seven of which records were made of the arterial pressure and respiration, rabbit blood was used in a dose of 1.5-5.9 ml/kg in order to produce tachphylaxis. Ox blood was infused 8 minutes 45 seconds -- 16 minutes 21 seconds after the injection of rabbit blood. In two of these experiments the injection of the small dose (2 and 5 ml/kg) diminished the shock reaction and the rats tolerated the injection of the lethal doses of ox blood. In the remaining 9 experiments no perceptible protective action was found and the rats died.

Thus, specificity of tachyphylaxis is observed in white rats but it is very relative. Consequently our experiments lead to the conclusion that the injection of small doses of ox blood into white rats evokes the development of protective and adaptational reorganization, preventing shock and acute edema of the lungs after the subsequent infusion of large doses of the same blood.

SUMMARY

The author has studied a number of medicines which prevented hemotransfusional shock and acute pulmonary edema in white rats. Preliminary intravenous administration of low doses (from 2 to 5 ml per kg of body weight) of cattle blood was the most effective. It was established that in only 3 minutes after the injection of low doses of blood the animal is protected from death, provided further injection of large doses of blood is effected. This phenomenon is relatively specific.

LITERATURE CITED

[1] A. D. Ado, Antigens as Extraordinary Stimuli of the Nervous System, AMN SSSR, Moscow (1952).*

[2] N. G. Belen'kii, A Nonspecies-specific Serum, Moscow (1950).*

[3] B. G. Veksner, Nov. Khirugrg. Arkh., 40, 1-2, 96-102 (1937).

[4] Idem, Ibid. 47, 3, 117-122 (1940).

[5] P. F. Zdrodovskii, The Present State of Experimental Immunology and Its Immediate Problems, Moscow (1956).*

[6] Iu. T. Koval', Khirurgiia, 7, 36-38 (1939).

[7] V. N. Krylov, Zhur. Mikrobiol. Epidemiol. i Immunobiol. 2, 67-71 (1947).

[8] Idem, Ibid., 1, 60-63 (1950).

[9] G. V. Kurygin, Iaroslavl Medical Institute. Collection of Scientific Papers in Celebration of the 10th Anniversary of the Institute, pp. 379-384, Iaroslavl (1954).*

[10] I. R. Petrov, A. N. Filatov, L. G. Bogomolova and K. V. Stroikova, Sovet. Khirurgiia, 7, 2-3, 410, 436 (1934).

[11] I. B. Studzinskii, The Toxic Properties of Blood, Kiev, (1913).*

* In Russain.

[12] A. N. Filatov, Blood Substitutes, Leningrad, (1944).*

[13] C. Champy and E. Gley, Compt. rend. Soc. de Biol. 1911, Vol. 71, p. 159-162; 420-432.

[14] Lambert, Ancel and Bovin, Compt. rend. Soc. de Biol. 1911, Vol. 71, p. 350-351, 415-416, 557-558, 720-723.

[15] H. Selye, J. of Clin. Endocrinol. 1946, Vol. 6, N. 2, p. 117-230.

[16] J. J. Wolfe, and C.R. Gupta, Brit. med. J. 1941, 4222, p. 807-808.

* In Russain.

THE EFFECT OF TRANSFUSION OF HOMOLOGOUS BLOOD AND OF BELEN'KII'S THERAPEUTIC SERUM (BTS) ON THE CONTENT OF ADRENALIN-LIKE SUBSTANCES IN THE HEART OF RABBITS

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The most characteristic and important feature of the mechanism of the stimulating action of various forms of blood transfusion is their effect on metabolism. We have postulated [2-8] that this effect is due to the action of different forms of blood transfusion on the tropic function of the nervous system and the glands of endocrine secretion. In previous reports [2-8] it was shown that transfusion of homologous blood and the primary transfusion of BTS alter the functional condition of the cerebral cortex, of all divisions of the vegetative nervous system and of the glands of internal secretion. These findings to some extent demonstrate the influence of various forms of blood transfusion on the trophic function of the nervous sytem and, consequently, it role in the changes in metabolism during various forms of hemotherapy.

In view of the importance of the problem we deemed it necessary to study it in the heart. Under these circumstances we set out from the following assumptions: the intensifying trophic nerve of I. P. Pavlov is sympathetic; the sympathetic nervous system possesses, according to L. A. Orbeli and others, adaptational and trophic functions; adrenalin-like substances are the mediators of sympathetic impulses. In accordance with the above we studied the influence of transfusions of homologous blood on the content of adrenalin-like substances in the heart of rabbits.

EXPERIMENTAL METHOD

Rabbits were transfused with freshly preserved (by formula 7 of the Central Institute of Blood Transfusion) rabbit blood in a volume of 7-10 ml per 1 kg body weight. At intervals of 30 minutes, 24 and 72 hours after the blood transfusion the animals were decapitated and the adrenalin-like substances in the hearts of the animals estimated by V. O. Osinskaia's method [11], as worked out in A. M. Utevskii's laboratory. This method makes it possible to estimate the noradrenalin (NAD), the adrenalin (AD) compounds with certain properties of oxidation products of adrenalin-like substances (OP) and "nonadrenalin fluorescence."

EXPERIMENTAL RESULTS

Observations were made on 60 rabbits (30 experimental, 15 control and 15 donors). Our investigations confirmed the findings of V. O. Osinskaia [11] that in the heart of the normal rabbit noradrenalin is present but not adrenalin. It was found that transfusion of homologous blood is accompanied by an increase in the noradrenalin in the heart of rabbits. This increase was observed 30 minutes, 24 hours (Fig. 1) and even 72 hours after the transfusion, when the noradrenalin content of the heart of the experimental animals was greater than that of the controls.

It is interesting to compare the findings with the results of investigations into the effect of transfusion of homologous blood on the heart. The method of electrocardiography is known to give very contradictory results.

Meanwhile clinical observations on the use of blood transfusion in patients with posthemorrhagic anemia have shown the extremely favorable influence of transfusion of homologous blood on the activity of the heart.

E. R. Gesse [1] pointed out: "Zeifert, Vedergake, Tseler et al. stress the importance of blood transfusion as a means of stimulating the activity of the heart to a much greater extent than any drug." What is responsible for this action of blood transfusion? In the opinion of the author, "the transfused blood acts as a replacement of the blood which has been lost, the nerve centers and cardiac ganglia emerge from their state of anoxemia, and the blood vessels are filled with fluid" [1].

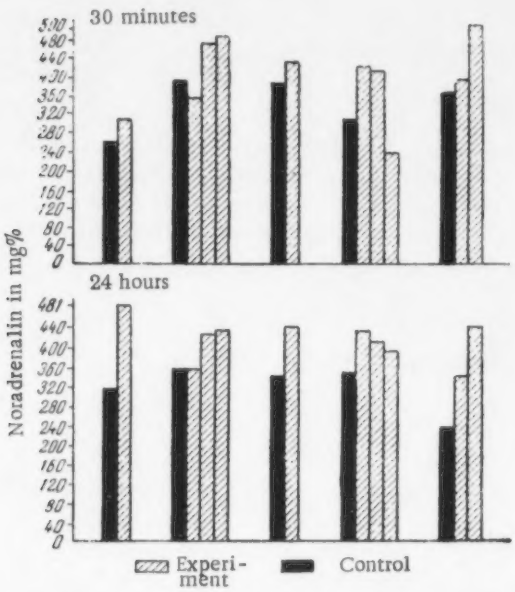

Fig. 1. The effect of transfusion of homologous blood on the noradrenalin content of the rabbit's heart.

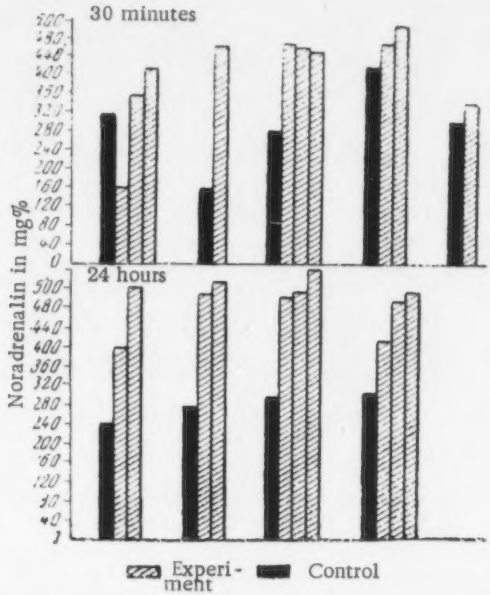

Fig. 2. The effect of primary transfusion of the heterogenic blood substutute BTS on the content of adrenalin-like substances in the heart of a rabbit.

In the literature to which we had access we could find no evidence for this assumption, and we decided to subject it to experimental analysis. If the increase which we found in the noradrenalin in the heart is brought about by some function of the transfused red cells, it must be absent after transfusion of plasma or serum; on the other hand, if it appears the effect which is produced must be due not to the replacement action of the red cells but to the stimulating action of the transfusion itself. For this reason in other experiments we studied the effect of primary transfusion of the heterogenic blood substitute BTS on the content of adrenalin-like substances in the heart of rabbits. Investigations carried out on 42 rabbits (30 experimental and 12 control) gave the same results in principle as with blood transfusion (Fig. 2).

The results described indicate that various forms of transfusion (blood, BTS) bring about an increase in the adrenalin-like substances in the heart of rabbits; this is new evidence of their influence on the trophic function of the nervous system and confirmation of the hypothesis which we have put forward previously of the single mechanism of action of the various forms of transfusion.

In the antishock effect of transfusions of blood and various blood substitutes the important role of the colloidal nature of the substance used may justly be pointed out. However to explain the whole affect on the grounds of colloidal properties would obviously be incorrect. Our findings demonstrate the importance of a cardiac factor which, as we know, plays an essential role in the maintenance of the level of the blood pressure. In this connection it is important to take note of findings which demonstrate the effectivenss of noradrenalin in the treatment of shock. There are grounds for assuming that the increase in the noradrenalin in the heart may to some extent

have a beneficial effect during intra-arterial transfusions of blood in cases of agony and clinical death, particularly if the possiblity of automatic stimulation of the function of the sympathetic ganglion by blood transfusion, as we have pointed out, is remembered.

Finally the results obtained enable us to put forward for discussion the question of rational transfusion during dystrophic conditions of the myocardium. In this respect the following comparison appears to be of some interest: V. O. Osinskaia [11] showed that in animals with severe experimental thyrotoxicosis the content of noradrenalin in the heart is sharply reduced. V. A. Oppel' [9] with his co-workers and other investigators observed the beneficial effect of transfusion of homologous blood on the cardiac activity of patients with a severe form of Basedow's disease (blood transfusion was given to these patients as a preoperative measure). Our results show that blood transfusion is accompanied by an increase in the noradrenalin in the heart. In other words, an increase in the noradrenalin content of the heart may coincide with an imporvement in its functional condition.

SUMMARY

The author established that isohemotransfusion and transfusion of Belenskii's therapeutic serum is associated with a pronounced and prolonged increase of the noradrenalin concentration in the rabbit's heart. This confirms the author's hypothesis on the single mechanism in the action of various types of hemotherapy and the role of the trophic function of the nervous system in the mechanism of their action. On the basis of the above data it is recommended to employ hemotherapy in dystrophic processes of the myocardium.

LITERATURE CITED

[1] E. R. Gesse, Vestnik Khirurg. im. Grekova, 50, 133-134, 36-41 (1937).

[2] R. M. Glants, Vrachebnoe Delo, 11, 983-986 (1953).

[3] Idem, Problems of Blood Transfusion, Vol. 4, pp. 7-18; 51-57, Kiev, 1955.*

[4] Idem, Transactions of the Eighth Congress of Surgeons of the Ukrainian SSR, pp. 376-378 and 506-507, Kiev, 1955.*

[5] Idem, Vrachebnoe Delo, 2, 111-116 (1955).

[6] Idem, Problemy Gematol. 3, 42-45 (1956).

[7] Idem, Proceedings of the 35th Plenum of the Central Institute of Hematology and Blood Transfusion, pp. 23-24.*

[8] Idem, Vrachebnoe Delo, 9, 921-924 (1956).

[9] V. A. Oppel', Lectures on Clinical Surgery and Clinical Endocrinology for Surgeons, Moscow-Leningrad, Vol. 2, (1931).*

[10] L. A. Orbeli, Lectures on the Physiology of the Nervous System, Moscow-Leningrad (1938).*

[11] V. O. Osinskaia, Biokhimiia, 3, 537-454 (1957).**

[12] F. Kaindl and A. Lindner, Wiener Ztschr. inn. Med. 1950, Bd. 31, S. 377-385.

[13] G. S. Kurland and M. Malach, New England J. Med., 1952, Vol. 247, pp.383-389.

[14] S. O. Liljedahl and O. Norlander, Svenska Läkartidn, 1951, Vol. 46,pp. 2762-2765.

[15] W. R. Liversay and Don W. Chapman, Am. J. Med. Sc. 1953, Vol. 225,pp. 159-171.

[16] A. J. Miller and L. A. Baker, Arch. Intern. Med. 1952, Vol. 89, p. 591-599.

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* In Russian.

** See English translation.

PER OS TREATMENT OF EXPERIMENTAL DIABETES MELLITUS

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At the beginning of 1957, in the Ukrainian Institute of Experimental Endocrinology, was synthesized 4-methylbenzenesulfanilylbutyl urea (T. F. Sysoeva and N. I. Makhnenko). In the Soviet Union this preparation is called butamid; abroad it is called D-860, orinase or tolbutamide (in the USA), rastinon or artosine (in Germany) and dolipol (in France). Like the preparation B-55 (nadisan, invenol, glucidoral or carbutamide) it possesses a hypoglycemic action on per os administration, but in contrast to nadisan it is practically without any antibacterial properties. Treatment of patients with diabetes mellitus lasts throughout life, and so the absence of antibacterial properties is an important advantage of butamid. It is, in addition, less toxic than nadisan.

As previous investigations (1957) have shown, butamide in a dose of 0.5 g per 1 kg body weight lowered the blood sugar of normal rabbits for 24 hours by 33-52%, while the blood sugar level in control animals fell by 8-10% and in dogs (in a dose of 100 mg per 1 kg body weight) – by 23 and 36% compared with 15 and 10% in control experiments. Butamid acted differently on the blood sugar of dogs with alloxan diabetes. In some animals the hypoglycemic sulfonamides lowered the blood sugar and in others its level was not altered. The same thing was observed in dogs with alloxan diabetes after injection of these animals with butamid. This effect evidently depends on the amount of residual islet tissue: the more it is damaged, the weaker the hypoglycemic action of the sulfonamides will appear.

We subsequently set out to discover whether butamid reinforces the action of insulin when injected from outside.

EXPERIMENTAL METHOD

Experiments were carried out on dogs with alloxan diabetes and subjected to total pancreatectomy, requiring considerable amounts of insulin. The animals spent the whole time in metabolism cages. Because of this, their daily dietary intake could be calculated and the excreted carbohydrate estimated, from which the utilized carbohydrate could be determined. The effectiveness of butamid was judged by the intensity of fall of hyperglycemia and glycosuria, by the value of utilized carbohydrate and by the amount of insulin which could be dispensed with by means of butamid. The blood sugar was determined by the Hagedorn-Jensen method, and the sugar in the urine polarimetrically. The investigation was carried out on 4 dogs with alloxan diabetes and receiving large amounts of insulin: 16, 24, 32, and 40 units per day. This quantity of insulin did not abolish the hyperglycemia and glycosuria. Butamid was injected into all the dogs in a dose of 100 mg per 1 kg body weight. 84 experiments were performed on each dog. In some experiments (control) the effects were studied on hyperglycemia, glycosuria and assimilation of carbohydrate of insulin alone, and in others – of insulin and butamid. Experiments of a similar type were carried out also on dogs subjected to total pancreatectomy. These received daily 150 g of raw pancreas in addition to their usual diet. We performed 134 experiments on the dog Mal'chik, and 66 on Pushok. Insulin was injected twice daily into the animals – at 9 a.m. and 4 p. m., and butamid – at 9 a.m. (except in a few experiments when it was injected at 2 p.m.).

EXPERIMENTAL RESULTS

The results in Table 1 demonstrate the considerable reinforcement by butamid of the insulin effect. The same results were obtained in comparable experiments on all 4 dogs.

Later on we attempted to discover the intensity of action of butamid – the amount of insulin which could be replaced by butamid. For this purpose the doses of insulin injected into the dogs were gradually reduced to values which, together with butamid, would give the same effect as large doses of insulin alone.

TABLE 1

The Effect of Butamid on Alloxan-Diabetic Dogs

Dogs	Sivka				Torba			
Data of experiments	13—22/VII	23—27/VII	14—20/IX	21—30/IX	13—22/VII	23—27/VII	11—15/IX	16—26/IX
Number of experiments	10	5	7	10	10	5	5	11
Dose of insulin, units	24	24+B *	24	24+B	24	24+B	36	36 + B
Hyperglycemia, in mg%	349	211	302	313	348	229	418	277
Glycosuria, g	45	5	68	44	32	3	37	4
Assimilation of carbohydrate, g	119	148	94	154	164	192	161	194

* In this and the subsequent tables, B – butamid.

The figures in Table 2 show that butamid reinforces the action of insulin by 25, 33 and 50%. This means that with the aid of butamid it is possible to reduce the insulin requirement of alloxan-diabetic dogs by 25-50% respectively.

What is the mechanism of reinforcement of the insulin effect by butamid?

The sulfanilamides are known not to influence the blood sugar level of animals from which the pancreas has been removed. On this basis Loubatières put forward the hypothesis that the sulfanilamides stimulated the β-cells of the insulin apparatus. This hypothesis was supported by several authors [1, 4, 5, 6, 9].

Although in the lowering of the blood sugar by sulphanilamides, depression of the glucose-6-phosphatase activity of the liver may possibly have some importance, together with a reduction in the absorption of glucose from the intestine and other factors, Holt et al. [1] nevertheless assert that the main role in this activity belongs to stimulation of the β-cells and not to extrapancreatic reinforcement of the insulin effect. Some authors have tried to prove the importance of suppression of the α-cells in the hypoglycemic effect of the sulfanilamides. However investigation of the factors quoted as evidence in favor of this action of the sulfanilamides failed to confirm them.

Nevertheless there appeared recently a paper by Schmidt and Blobel [10], which showed that under the influence of D-860, rats always showed a reduction (statistically significant) in the nuclear volume of the α-cells of the insulin apparatus.

If depression of the α-cells is really of importance in the hypoglycemic effect of the sulfanilamides, it is not clear why butamid, like nadisan, does not affect the blood sugar level in animals with severe alloxan diabetes. It is not clear why the effect of sulfanilamides is not shown in patients with severe diabetes mellitus. From the point of view of the importance of the β-cells in the hypoglycemic effect of these preparations, its absence in some of them may be due to the small number of β-cells which are left or to severe weakening of their function.

But how do we explain the reinforcement of the insulin effect in all the alloxan-diabetic animals? It seemed to us that this effect in the dogs with severe alloxan diabetes depends not on the action of butamid on the pancreas but on its action on the insulin effect itself. We verified this hypothesis on totally depancreatized dogs receiving a definite amount of insulin which did not abolish the hyperglycemia and glycosuria (Table 3).

TABLE 2

Intensity of the Action of Butamid on Alloxan-Diabetic Dogs

Dogs	Aza			Kutska			Sivka		Torba	
Date of experiments	13—18/VII	20—22/VII	23—27/VII	13—21/VII	16—20/IX	21—30/IX	13—22/VII	28/VII—3/VIII	11—15/IX	13—22/VII
Number of experiments	6	3	5	9	5	10	10	7	5	5
Dose of insulin, units	32	16	16+B	16	12	12+B	24	16+B	36	24+B
Hyperglycemia, mg%	209	284	195	207	342	244	349	175	418	229
Glycosuria, g	8	71	8	6	63	10	45	4	37	3
Assimilation of carbohydrate, g	141	105	127	141	130	165	119	128	161	192

The results in Table 3 show that additional injections of butamid considerably reinforce the insulin effect in totally depancreatized animals. Thus, after injection of 40 units of insulin with butamid to the dog Mal'chik, the hyperglycemia fell from 298 to 123 mg%, the glycosuria from 9 to 0 g, and the carbohydrate assimilation increased from 92 to 114 g. After injection of butamid with 20 units of insulin the hyperglycemia fell from 339 to 233 mg% and the glycosuria from 33 to 8 g, and the carbohydrate assimilation rose from 71 to 136 g. From Table 3 it can also be seen that butamid sharply reinforced the insulin effect on the dog Pushok also. Table 3 further shows that butamid in the dog Mal'chik can reduce the insulin requirement by 20 units. Butamid will replace the same amount of insulin in the dog Pushok also.

After the conclusion of the present investigation we became aware of the papers of Sirek and Sirek [11] and by Campbell. These workers also found it possible to replace a considerable amount of insulin in totally depancreatized animals by nadisan — 50-75%, and in one dog they reduced the insulin intake to 1 unit for a period of 8-10 days. On the other hand, Sirek and Sirek [11] were unable to obtain any reaction to injection of nadisan (in addition to insulin) in one dog after total removal of the pancreas.

Considering the large number of experiments which we performed on 6 diabetic dogs, and also the findings of other workers, it may be concluded that nadisan and butamid considerably reinforce the insulin effect after total removal of the pancreas.

Attention is drawn to the fact that in our experiments butamid reinforces the insulin effect in alloxan-diabetic and totally depancreatized dogs to almost the same intensity — by 25-50%. On this basis it can be assumed that the action of butamid on alloxan-diabetic dogs is mainly expressed as reinforcement of the insulin effect and not as stimulation of the β-cells of the pancreas. It is possible also that the effect of butamid on the blood sugar level in normal animals depends not only on the stimulation of the secretion of insulin by the pancreas but also on reinforcement of the action of the insulin. Although the ability of butamid to stimulate the activity of the β-cells has been convincingly proved, the almost identical intensity of reinforcement of the insulin effect in alloxan-diabetic and totally depancreatized dogs gives rise to doubt of the geat importance in the mechanism of action of butamid of stimulation of the β-cells by the drug.

TABLE 3

The Effect of Butamid on the Metabolism of Totally Depancreatized Dogs

Dogs	Mal'chik				Pushok	
Duration of diabetes mellitus	over 8 years				over 4 years	
Date of experiments	28/V—12/VI	13—21/VI	6—18/VII	19—21/VII	4—17/VIII	18—28/VIII
Number of experiments	15	9	13	3	14	11
Dose of insulin, units	40	40+B	20+B	20	26	12+B
Hyperglycemia, mg%	298	123	233	339	363	322
Glycosuria, g	9	0	8	33	21	30
Assimilation of carbohydrate	92	114	136	71	82	81

How may the effect of butamid on insulin activity be represented in the absence of the pancreas? Even recently attempts were made to explain the action of butamid as weakening the activity of insulinase [7, 8]. However it was quickly shown that the action of butamid on insulin is apparent only at very high concentrations, and in therapeutic doses it does not affect the rate of destruction of insulin in the body to any great extent. The possibility is not excluded that in the presence of butamid, glycogen formation is intensified under the influence of insulin. It follows from the experiments discussed that the main action of butamid is to reinforce the insulin effect.

SUMMARY

It was shown by experiments on dogs with alloxan diabetes and those completely depancreatized that the amount of insulin required for the maintenance of a definite condition of diabetes mellitus is considerbly decreased (by 25-50% under the effect of butamide, synthetized in the Ukrainian Institute of Experimental Endocrinology).

This definite condition of diabetes mellitus was evalutated by the level of hyperglycemia, glucosurea and the quantity of the absorbed carbohydrates. There was almost an equal potentiation of insulin action by butamide in both groups of animals. Thus, a conclusion was drawn that the main effect of butamide consists in the potentiation of the insulin effect.

LITERATURE CITED

[1] C. Holt, L. Holt and B. Krõner, Naturwissenschaften, 1956, Bd. 43, S. 162-163.

[2] J. Kracht and J. G. Rauch-Stroomann, Ibid. 1956, Bd. 43, S. 180-181.

[3] J. Kracht, C. V. Holt and L. V. Holt, Endocrinologie, 1957, Bd. 34, S. 129-145.

[4] J. Kracht, B. Krõner, L. Holt and C. Holt, Naturwissenschaften, 1957, Bd. 44, S. 16-17.

[5] A. Loubatières, Arch internat. de Physiol. 1946, v. 54, p. 174.

[6] Idem, Presse med. 1955, v. 63, p. 1701-1703.

[7] I. A. Mirsky, G. Perisutti and D. Diengott, Metabolism, 1956, v. 5, p. 156-160.

[8] I. A. Mirsky, G. Perisutti and R. Jinks, Proc. Soc. Exper. Biol. a Med. 1956, v. 91, p. 475-477.

[9] I. C. Rausch-Strooman and J. Kracht, Verhandl. dtsch. Ges. inn. Med. 1956, Bd. 62, S. 518.

[10] R. Schmidt and R. Blobel, Endocrinologie, 1957, v. 34, p. 155-161.

[11] A. Sirek, and O. Sirek, Canad. M. A. J. 1956, v. 74, p. 450.

THE EFFECT OF PENICILLIN ON THE BLOOD SYSTEM

COMMUNICATION II. THE CHARACTER OF THE LEUCOCYTE REACTIONS IN ANIMALS DURING PROLONGED ADMINISTRATION OF PENICILLIN

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As our investigations have shown, penicillin, when administered to healthy persons and animals, produces no essential changes in the peripheral blood but in a number of cases it accelerates the maturation of eosinophils in the bone marrow. In addition, reports in the literature and our own personal experience clearly indicate a comparatively high incidence of eosinophilia during penicillin therapy. In this connection we decided to study the character of the leucocyte reactions to various stimuli during the prolonged administration of penicillin. As suitable stimuli we used the intramuscular injection of 3 ml of skimmed milk. According to N. I. Veshchezerov, R. I. Volynskaia and A. Ia. Iaroshevskii [1, 2, 3], in cats the injection of milk always causes a pronounced neutrophilic leucocytosis with a marked shift in the leucocytic formula to the left. Another stimulus was distension of the stomach of cats through a fistula; it is well known at the present time that this type of manipulation leads, after a transient leucopenia, to leucocytosis without any marked changes in the leucocytic formula. Thus, the use of the first stimulus combined both nervous and humoral factors, whereas stretching of the mechanoreceptors of the stomach is an example of reflex action.

EXPERIMENTAL METHOD

At the beginning of the experiments the leucocyte reaction to the intramuscular injection of milk or to stimulation of the stomach was studied on two occasions in two weeks; during the next 14-28 days an injection of 50,000 units of penicillin was given every 4 hours, and the stimuli were repeated on the 7th-9th-14th and 27th days of administration, and also a week after the injections had been discontinued. Blood was taken from the ear of the animals before stimulation and 30 minutes, 1, 2, 3 and 4 hours after stimulation.

Althogether 78 experiments were carried out on 12 cats (in four of these animals a gastric fistula was created 3 months beforehand).

EXPERIMENTAL RESULTS

In 6 our of 8 animals, in response to the injection of 3 ml of milk a marked leucocytosis was observed, appearing usually after a transient ($\frac{1}{2}$ -1 hour) leucopenia. The maximum increase in the white cell count took place in the 2nd and 3rd hours of the experiment. After only 2 hours a marked shift was observed in the leucocytic formula with an increase in the content of stab cell forms to 9-16%.

The content of eosinophils under these circumstances was essentially unchanged (Fig. 1, a and b and Fig. 2). It can be seen from the Table that in two experiments (November 13 and 26) carried out before administration of

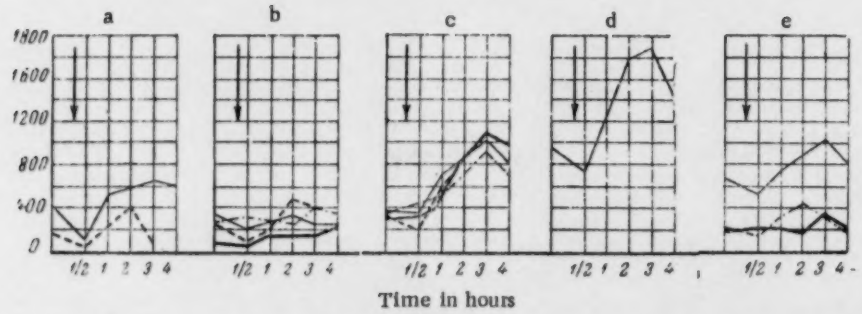

Fig. 1. Changes in the numberof eosinophils during penicillin administration after injection of milk.
a, b) Before giving penicillin; c) on the 2nd day of penicillin administration; d) on the 7th day of penicillin administration; c) 9 days after discontinuing penicillin. The arrows indicate injection of milk.

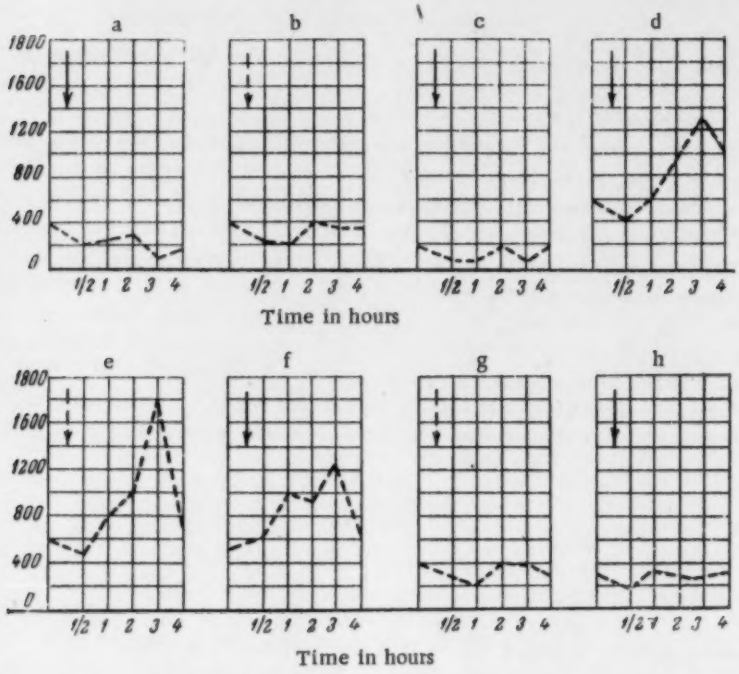

Fig. 2. Changes in the eosinophil content after injection of milk and distension of the stomach.
a, b, c) Before injection of penicillin; d) on the 10th day after injection of penicillin; e) on the 16th day; f) on the 28th day of penicillin administration; g-h) on the 8th-9th days after discontinuing penicillin; ↓ – injection of milk; ⇣ – distension of stomach.

penicillin, during investigation of the blood a shift of the leucocytic formula to the left, without any notable increase in the eosinophil content, was clearly observed in response to the injection of milk. After preliminary injections of penicillin (see Table – experiments on December 10 and 28, Fig. 1, c and d and Fig. 2), on the 2nd and 7th days the injection of milk leads to a sharp increase in the eosinophil content; 9 days after discontinuing

TABLE

Changes in the Character of the Leucocyte Reactions to Milk After Administration of Penicillin. Experiment Dated November 13, 1953

Time in hr and min	Hemoglobin (in %)	Red cells	White cells	Eosinophils	Stab cells	Segmented neutrophils	Lympho-cytes	Monocytes	Absolute eosinophil count
Before administration of penicillin									
12^{15}	59	4 440 000	15 100	2,5	4	70	18	5,5	377,5
12^{21}	2.5 ml of milk injection intramuscularly								
12^{51}	60	4 580 000	8 200	2	4	72	12	10	164
13^{21}	59	4 490 000	16 200	1,5	12	69,8	12,0	5	243
14^{21}	58	4 570 000	21 900	1,5	14,5	65	13	6	328,5
15^{21}	59	4 840 000	19 100	0,5	10,5	69,5	13,5	6	95
16^{21}	60	4 950 000	15 400	1	7	71	14,5	6,5	154
Experiment dated November 26, 1953									
12^{01}	59	4 300 000	13 100	1,5	4	71,5	17,5	5,5	196,5
12^{18}	2.5 ml of milk injected intramuscularly								
12^{43}	61	4 540 000	9 600	1	4	74,5	16	4,5	96
13^{12}	62	1 490 000	13 700	0,5	4,5	78,5	12	4,5	68,5
14^{12}	61	4 430 000	19 950	1	10	76	9,5	3,5	199,5
15^{12}	60	4 470 000	14 260	0,5	12	74,5	8,5	4,5	71,3
16^{12}	61	4 510 000	14 100	1,5	12,5	71	12,5	2,5	211,5
Experiment dated December 10, 1953. On the 9th day of penicillin administration									
12^{03}	59	4 500 000	15 200	2	3,5	67,5	20,5	6,5	304
12^{10}	2.5 ml of milk injected								
12^{40}	62	4 940 000	11 200	4	3	66,5	19,5	7	448
13^{10}	60	4 750 000	11 350	5	4,5	66,5	17,5	6,5	567,5
14^{10}	61	4 750 000	18 500	5	6	71	12	6	925
15^{10}	62	4 860 000	21 900	6	10	66	11,5	6,5	1 314
16^{10}	59	4 520 000	17 000	6	10	67,5	10,5	6	1 020
Experiment dated December 28, 1953. On the 27th day of penicillin administration									
12^{16}	56	4 370 000	15 400	3,5	4	69,5	17,5	5,5	539
12^{21}	2.5 ml of milk injected into the paw.								
12^{51}	57	4 470 000	13 900	4,5	3,5	69,5	16,5	6	625,5
13^{21}	58	4 560 000	15 100	6,5	6	65,5	15,5	6,5	981,5
14^{21}	58	4 600 000	15 270	6	8,5	62,5	15,5	7,5	916,2
15^{21}	60	4 720 000	18 900	6,5	11	60	17,5	5	1228,5
16^{21}	59	4 620 000	16 100	4	10,5	67	13	5,5	644

the injections of the antibiotic the reaction to milk once more returns closer to its original pattern (Fig. 1, e). In order to avoid the influence of the repeated injections of a foreign protein themselves, in two cases we injected milk from an animal receiving penicillin for 2 weeks without preliminary injection of milk, and in this case too eosinophilia was observed.

Analogous findings were obtained also in experiments in which the stomach was distended by means of a thin-walled rubber balloon inserted within its lumen (Fig. 2 and 3); the pressure inside it was usually 35-40 mm of mercury. In Fig. 3 it may be seen that on the 15th and 22nd days of administration of penicillin, stumulation of the mechanoreceptors of the stomach leads to a significant eosinophilia, disappearing on the 9th-10th day after discontinuing the injection of the antibiotic.

The results of our experiments show that penicillin causes a state of sensitization in which the ordinary leucocyte reactions to humoral and reflex stimulation are modified and acquire the character of eosinophilia.

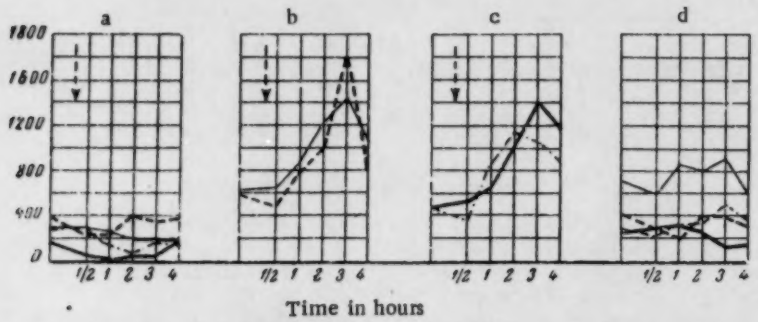

Fig. 3. Changes in the eosinophil content after distension of the stomach. a) Before administration of penicillin; b) on the 15th day of administration of penicillin; c) on the 22nd day of administration of penicillin; d) on the 9th-10th day after discontinuing penicillin.

Thus, in those cases in which the animals (cats) were given preliminary injections of penicillin, an injection of foreign protein in the form of milk causes eosinophilia instead of the usual neutrophilic leucocytosis.

Stimulation of the mechanoreceptors of the stomach in cats receiving injections of penicillin also leads to an increase in the number of eosinophils in the peripheral blood.

SUMMARY

In response to milk injection or to stomach inflation leukocytosis develops in cats following a short period of leukopenia. There is also a shift of the leukocytic formula with an increase of the number of band forms without any rise in the number of eosinophilic elements. Preliminary administration of penicillin, injection of foreign protein (milk) or the stimulation of the gastric mechanoceptors caused a pronounced eosinophilia disappearing in 9-10 days after discontinuing the administration of this antibiotic. Thus, penicillin causes a condition of sensitization in which the usual leukocytic reactions to humoral and the reflex stimulations are distorted and acquire the character of eosinophilia.

LITERATURE CITED

[1] N. I. Veshchezerov, The Mechanisms of Pathological Reactions, 3, pp. 34-40, Leningrad (1941).*

[2] R. I. Volynskaia, The Mechanism of Leucocytosis, Dissertation, Leningrad (1946).*

[3] A. Ia. Iaroshevskii, Interoceptors of the Bone Marrow and Interoceptive Influences on the State of the Blood. Dissertation, Leningrad (1948).*

* In Russian.

BIOCHEMISTRY AND BIOPHYSICS

QUANTITATIVE ASPECTS OF RESPIRATION AND GLYCOLYSIS IN THE WHITE CELLS OF HUMAN BLOOD

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In our previous investigations we have studied in detail the processes of energy exchange in the white cells of human blood [2, 3]. It was found that the energy exchange of the granulocytes is distinguished by three characteristic features: aerobic glycolysis, a reverse Pasteur reaction (inhibition of respiration by glycolysis) and complete resynthesis of adenosinetriphosphate (ATP) in both aerobic and anaerobic conditions. All these three metabolic features have previously been found in cancer cells [1]. In this way it was found that there was a qualitative resemblance between leucocytes and malignant tumors in certain important processes of cell metabolism.

In both his early and recent work on cancer, Warburg regards the respiration of cancer cells as "incomplete" in both the qualitative and quantitative sense, and attaches particular importance to the relationship between glycolysis and respiration. According to Warburg, for neoplastic tissues the ratio $Q^{O_2}_{CO_2} / Q_{O_2} = 3\text{-}4$, whereas for normal tissues (including growing tissues in which there is aerobic glycolysis) it does not exceed 1 [4].

Since the leucocytes showed, as pointed out above, such a profound similarity with malignant tumors in a number of fundamental processes of energy exchange, it appeared interesting to make an accurate quantitative analysis of the signs of qualitative resemblance of leucocytes and malignant cells which have been observed. Under these circumstances particular attention would have to be paid to the metabolic characteristics which are regarded by Warburg as specific for neoplastic tissues. For this purpose we determined the absolute values of respiration and glycolysis in the leucocytes in optimal conditions for these two processes, and calculated the ratio between the activities of respiration and glycolysis in each type of white blood cell.

EXPERIMENTAL METHOD

Human white blood cells, practically free from red cells and platelets, were suspended in blood serum at 37°C in the vessels of a Warburg apparatus. Respiration was recorded manometrically – glycolysis photometrically with p-hydroxydiphenyl. In order to detect the respiratory and glycolytic activity of the cells in optimal conditions, in our present investigation in contrast to the previous ones, we used suspensions with smaller concentrations of cells – 0.036 ml of leucocytes per 1 ml of experimental suspension (alongside the previously used concentrations). In this way maximum relative velocities of respiration and glycolysis were attained. Special experiments showed that the relative velocities of these two processes increase with increasing dilution of the leucocyte suspensions, reaching a stable maximum value of activity at cell concentrations of 0.04-0.05 ml per 1 ml of experimental suspension.

EXPERIMENTAL RESULTS

In the Table are given the results of the study of the rates of respiration and glycolysis in the different groups of human white blood cells in normal and some pathological conditions. The respiratory and glycolytic activities

TABLE

Respiration, Glycolysis and Their Ratio in Various Forms of Human Leucocytes

Leucocytes	0.03 ml of cells per 1 ml of experimental suspension					0.1 ml of cells per 1 ml of experimental suspension				
	$Q^{glucose}_{O_2}$	$Q^{air}_{CO_2}$	$Q^{N_2}_{CO_2}$	$\frac{Q^{air}_{CO_2}}{Q_{O_2}}$	$\frac{Q^{N_2}_{CO_2}}{Q_{O_2}}$	$Q^{glucose}_{O_2}$	$Q^{air}_{CO_2}$	$Q^{N_2}_{CO_2}$	$\frac{Q^{air}_{CO_2}}{Q_{O_2}}$	$\frac{Q^{N_2}_{CO_2}}{Q_{O_2}}$
Donors'	5,7±0,3	18,8±1,0	31±1,1	3,3	5,5	2,6±0,1	6,0±0,5	8,8±0,6	1,8	3,4
Chronic myeloid leukemia	5,0±0,3	13,2±0,7	23,[illegible]±0,8	2,6	4,7	3,1±0,2	5,6±0,2	8,9±0,8	1,8	2,9
Chronic lymphtic leukemia	7,9±0,5	0	27,2±1,0	—	3,4	6,6±0,3	0	11,1±0,8	—	1,7

are expressed as Warburg coefficients: Q_{O_2}, $Q^{O_2}_{CO_2}$, $Q^{N_2}_{CO_2}$ (in mm^3 gas per 1 mg dry weight of cells per hr).

It follows, first, from the Table that the metabolic indices depend to a large extent on the concentration of cells per unit volume of experimental suspension. In suspensions with a concentration of 0.03 ml of cells per 1 ml of experimental suspension, the respiration and glycolysis are as a rule significantly higher than in a concentration of cells of 0.1 ml per 1 ml of experimental suspension. The respiration of lymphocytes constitutes an exception to this for it changes only a little with changes in the cell concentration (Q_{O_2} equal to 7.9 and 6.6).

The figures given in the Table of respiration and glycolysis of leucocytes in optimal conditions (low concentration of cells, serum at 37°C) clearly show one of the most important laws of metabolism of the white blood cells — comparatively low respiration and very high glycolysis. This holds good even for aerobic cells — lymphocytes — in which, despite the absence of lactic acid formation in aerobic conditions, the anaerobic glycolysis is very high.

Normal donors' leucocytes absorb on the average 5.7 mm^3 O_2 calculated per 1 mg dry weight of cells per hour. At the same time they excreted under anaerobic conditions a quantity of lactic acid equivalent to 31 mm^3 CO_2 per 1 mg dry weight per hour. In aerobic conditions 18.8 mm^3 CO_2 was given off. Corresponding figures obtained for white blood cells of patients with chronic myeloid leukemia showed indices of respiration and glycolysis which were close to those of normal leucocytes: $Q_{O_2} = 5$; $Q^{air}_{CO_2} = 13.2$; $Q^{N_2}_{CO_2} = 23.3$. Calculation of the ratio $Q^{N_2}_{CO_2} / Q_{O_2}$ and $Q^{air}_{CO_2} / Q_{O_2}$ for normal leucocytes gives values of 5.5 and 3.3; for leucocytes of patients with chronic myeloid leukemia these figures were 4.7 and 2.6 respectively, and for lymphocytes, in which aerobic glycolysis does not occur and for which only the first coefficient may be calculated, the ratio $Q^{N_2}_{CO_2} / Q_{O_2}$ is 3.4.

The figures obtained acquire considerable importance when they are compared with the corresponding indices for cancer tissues. According to Warburg and his co-workers [4], epithelial carcinomas in man are characterized by the following values for respiration and glycolysis: $Q^{N_2}_{CO_2} = 41$; $Q^{O_2}_{CO_2} = 28$; $Q_{O_2} = 10.2$ (with corrections for "contamination" with connective tissue cells). For human sarcoma the same authors quote the

following figures: $Q^{N_2}_{CO_2} = 35$; $Q^{O_2}_{CO_2} = 19.5$; $Q_{O_2} = 6.1$ (also calculated per 100% of malignant cells). As will be seen, the metabolic indices are of the same order as those which we obtained for human leucocytes.

In his very first papers on metabolism of malignant tumors, Warburg attached particular importance to the ratio $Q^{O_2}_{CO_2} / Q_{O_2}$, considering that a value of 3-4 for this coefficient was specific for a neoplasm. For the results given in the Table, it follows that this requirement is satisfied not only by malignant tumors but also by the white blood cells of a healthy person, in which the ratio $Q^{air}_{CO_2} / Q_{O_2} = 3.3$. In later papers on cancer [5], Warburg stresses the importance of the maximum values of glycolysis of tumors, when comparing the respiration of malignant cells with their anaerobic glycolysis. If the ratio of anaerobic glycolysis to respiration is calculated for Warburg's experiments with human carcinomas and sarcomas, the corresponding figures obtained are 5 and 5.8. As may be seen from the Table, the ratio $Q^{N_2}_{CO_2} / Q_{O_2}$ for human leucocytes is 5.5. Consequently there is no difference between malignant tumors and leucocytes in this ratio also. For ascitic carcinoma cells of mice, which Warburg regards as a classic object for biochemical tumor research, he obtained the following metabolic characteristics: $Q_{O_2} = 5\text{-}10$; $Q^{O_2}_{CO_2} = 25\text{-}35$; $Q^{N_2}_{CO_2} = 50\text{-}70$. The coefficient $Q^{N_2}_{CO_2} / Q_{O_2}$ in this case is approximately 8, with possible variations on either side. The last figures, however, are not fully indicative, since they relate to mouse cells and not to human cells, and cannot be used in calculations to make comparisons with human leucocytes.

Simple comparison of the findings in the Table on the respiration and glycolysis of human leucocytes with those obtained by Warburg for human malignant tumors indicates the absence of any quantitative difference between the absolute levels of respiratory and glycolytic activity of these two categories of cells, and also the absence of any difference between the ratios of the values $Q^{N_2}_{CO_2} / Q_{O_2}$ and $Q^{O_2}_{CO_2} / Q_{O_2}$.

The facts presented speak unanimously in support of the view that neither the old concept of Warburg, which rests on aerobic glycolysis, nor the new variant, based on the recognition of the specificity of low respiration and high anaerobic glycolysis for malignant tumors, can be regarded as valid in the light of these new findings.

SUMMARY

The study of the velocity of respiration and glycolysis of various forms of human leukocytes deomonstrated that these indicators of metabolism of white blood cells may be quantitatively compared with these indicators found in case of human carcinoma and sarcoma (formerly established by O. Warburg and his collaborators. Coefficients $Q^{N_2}_{CO_2} / Q_{O_2}$ and $Q^{O_2}_{CO_2} / Q_{O_2}$ were also found to be very similar in cancer cells and leukocytes. In case of leukocytes of normal blood Q_{O_2} (5.7); $Q^{O_2}_{CO_2} = 18.8$; $Q^{N_2}_{CO_2} = 31$; the ratio $Q^{N_2}_{CO_2} / Q_{O_2} = 5.5$; the ratio $Q^{O_2}_{CO_2} / Q_{O_2} = 3.3$.

As to the leukocytes of patients with chronic myeloid leukemia these values equalled: 5, 13, 2; 23, 3; 4.7 and 2.6, respectively. In case of lymphocytes the values of respiration and glycolysis were: $Q_{O_2} = 7.9$; $Q^{O_2}_{CO_2} = 0$; $Q^{N_2}_{CO_2} = 27.2$. The ratio $Q^{N_2}_{CO_2} / Q_{O_2} = 3.4$.

LITERATURE CITED

[1] N. V. El'tsina and I. F. Seits, Doklady Akad. Nauk SSSR, 77, 4, 653-656 (1951).

[2] I. S. Luganova, I. F. Seits and V. I. Teodorovich, Ibid. 112, 6, 1032-1035 (1957).

[3] Idem, Voprosy Med. Khim. 3, 6, 428-439 (1957).

[4] O. Warburg, F. Negelein and K. Posener, Biochem. Ztschr.. 1924, Bd. 152, S. 115-28.

[5] O. Warburg, Science, 1956, V. 123, p. 309.

PECULIARITIES OF THE DISTRIBUTION OF THE HYDROCHLORIDE OF THE DIETHYLAMINOETHYL THIOESTER OF DIPHENYLHYDROXYTHIOACETIC ACID IN THE BODY

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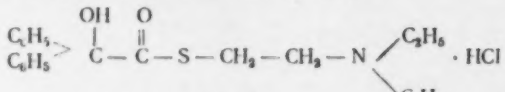
A. M. Kats [1] investigated the character of the distribution of the hydrochloride of the diethylaminoethyl thioester of diphenylhydroxythioacetic acid (thioester) in the body in various animals and studied the routes and the rate of its excretion from the body after intramuscular injection:



thioester

The aim of the present work was to study the peculiarities of the distribution of thioester in the body when administered in different ways, and to investigate the stability of its interaction with individual biochemical structures in the tissues.

EXPERIMENTAL METHOD

For this investigation, thioester labeled with radioactive sulfur (S^{35}) was synthesized.*

As experimental animals cats weighing 2-3 kg were used. In the work, the preparation was injected in various ways: intravenously (into the femoral vein) and intra-arterially (into the carotid and vertebral arteries). The preparation was injected as a 1% aqueous solution in a dose of 1-5 mg/kg. After predetermined intervals of time the animals were decapitated, the organs extracted, cleaned of blood and fat and homogenized in alkali by heating.

In fractionation experiments after the intravenous injection of thioester or the direct addition of the preparation to the finely chopped tissue, the organs were frozen in liquid oxygen and ground to the consistency of a fine powder, after which weighed samples were treated with 5% trichloroacetic acid (TCA). These were centrifuged and the centrifuge washed 10 times with 5% TCA. The centrifugates were discarded, and the precipitate, containing proteins and lipids, were extracted successively with alcohol, a mixture of alcohol and ether during which it was heated, and ether. The combined centrifugates comprised the lipid fraction. The precipitate is the protein fraction, and was freed from traces of ether by heating on a water bath. The radioactivity was measured in the tissue homogenate and separately in the lipid and protein fractions.

A. M. Kats showed that thioester does not undergo any perceptible decomposition in the body, and the radioactivity measured in the tissues shortly after the injection of thioester (S^{35}) belongs to the intact molecule of the

* The bulk of the preparation was synthesized by Z. I. Bobysheva, to whom we express our deep gratitude.

compound. For this reason we expressed the results of our experiments in μg of thioester, for which each time we measured the radioactivity of the solutions of thioester injected into the animals.

EXPERIMENTAL RESULTS

With the aim of studying the peculiarities of distribution of thioester when injected by various routes, we carried out several series of experiments. In the first series the preparation was injected intravenously in a dose of 1 mg/kg and the distribution of the thioester after different intervals of time was investigated. The results are given in Fig. 1, from which it can be seen that after 5 minutes the highest concentration of the preparation is found in the lungs. In the liver and kidneys it is considerably lower. At the end of the second hour the thioester concentration in the divisions of the brain, the kidneys and the lungs falls sharply. In the liver the content of S^{35} increases in the course of time.

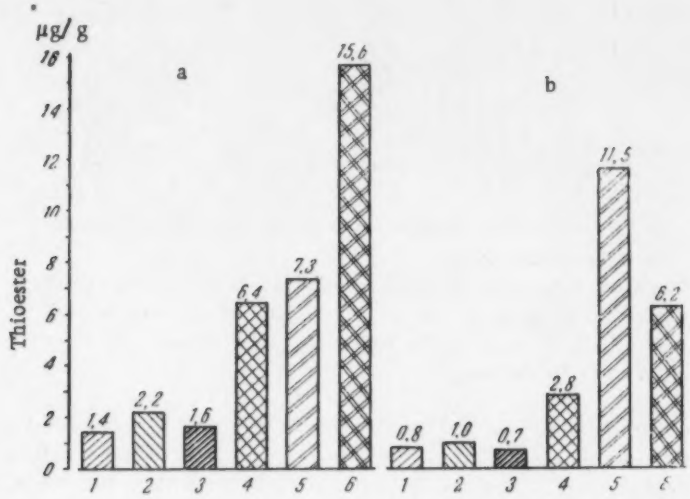


Fig. 1. Distribution of thioester in the cat after intravenous injection of the compound.
a) After 5 minutes; b) after 2 hours. 1) in the medulla; 2) in the cerebral hemispheres; 3) in the blood plasma; 4) in the kidneys; 5) in the liver; 6) in the lungs.

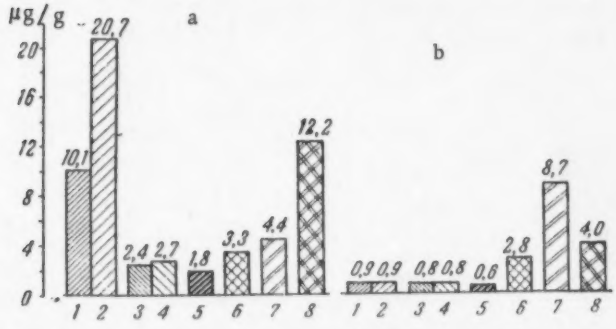


Fig. 2. Distribution of thioester in the cat after injection of the preparation into the right vertebral artery.
a) After 5 minutes; b) after 2 hours. 1) in the medulla on the left; 2) the same on the right; 3) in the left cerebral hemisphere; 4) the same on the right; 5) in the blood plasma; 6) in the kidneys; 7) in the liver; 8) in the lungs.

In the second and third series of experiments, thioester in a dose of 1 mg/kg was injected into the blood vessels supplying the brain.

The results are shown in Figs. 2 and 3. In Fig. 2 it can be seen that 5 minutes after its injection into the right vertebral artery the highest concentration of the preparation is found in the right half of the medulla. After 2 hours the thioester content of the medulla falls sharply and it becomes the same in all parts of the brain.

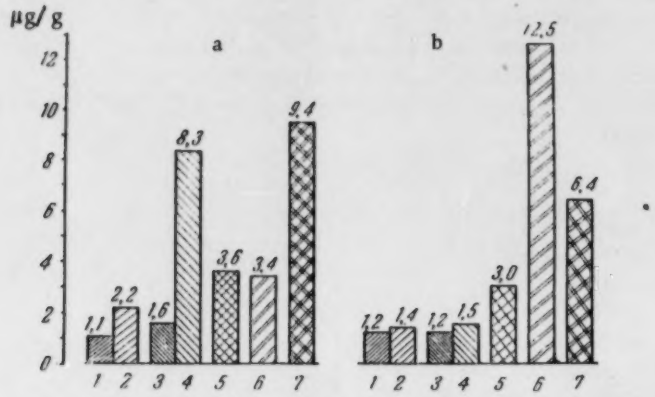


Fig. 3. Distribution of thioester in the cat after injection into the right carotid artery.
a) After 5 minutes; b) after 2 hours; 1) in the medulla on the left; 2) the same on the right; 3) in the left cerebral hemisphere; 4) the same on the right; 5) in the kidneys; 6) in the liver; 7) in the lungs.

The vertebral artery is known to enter the inferior arch of the circle of Willis and to supply mainly the brainstem. After injection into the carotid artery (Fig. 3) in the first few minutes a high concentration of thioester is created in the right cerebral hemisphere. In this case too, after 2 hours the content of the preparation in the parts of the brain becomes evened out at a low value. At the same time the content of thioester in the liver rises. In separate experiments we determined the concentration of thioester in the blood plasma. It was found to be low and practically the same after all ways of injection, nor did it change much with the course of time.

TABLE 1

Distribution of Thioester Among the Chemical Fractions of the Tissues of the Cat After Intravenous Injection of 5 mg/kg (mean results of 5 experiments; the figures expressed the radioactivity of the fractions as percentages of the total radioactivity of the homogenates)

Fractions / Organs	After 5 minutes		After 2 hours	
	protein	lipid	protein	lipid
Brain	12,14	25,71	Traces	16,63
Kidneys.	7,94	21,53	»	8,14
Liver	4,95	7,21	3,59	9,50
Lungs.	6,90	27,81	5,71	14,60

Thus, the study of the distribution of thioester after its injection by different routes into the body showed that shortly after injection of the preparation a high concentration is created in individual organs, but very soon a redistribution of the compound takes place. This peculiarity of the distribution of thioester may most probably

TABLE 2

Distribution of Thioester Among the Chemical Fractions of the Brain in Experiments in Vitro (incubation for 2 minutes)

Experiment No.	Concentration of thioester				
	in the homogenate (in μg/g)	in the protein fraction		in the lipid fraction	
		in μg/g	in %	in μg/g	in %
1	52,0	1,65	3,17	1,75	3,35
	304,0	11,40	3,75	1,85	0,61
	1925,0	94,50	4,91	14,00	0,73
2	32,0	1,96	6,13	1,03	3,22
	220,0	9,75	4,43	3,80	1,73
	2220,0	79,30	3,57	19,20	0,86
3	33,5	1,48	4,42	0,22	0,66
	190,0	8,21	4,32	2,17	1,14
	1870,0	72,00	3,85	15,80	0,84
4	30,8	1,65	5,36	1,05	3,41
	234,0	9,50	4,06	1,45	0,62
	2080,0	59,50	2,86	11,50	0,55
5	35,7	2,50	7,00	0,22	0,62
	242,0	10,80	4,46	1,43	0,59
	2060,0	85 70	4,16	16,60	0,81

TABLE 3

Content of Thioester (in μg/g) in the Protein Residue Before and After Dialysis

Experiment No.	Before dialysis	Time after dialysis (in hours)							%-clearance after 24 hours
		after 2	after 4	after 6	after 24	after 48	after 72	after 96	
1	61,0	53,7	44,8	49,3	18,35	14,0	—	—	69,9
2	58,6	43,7	37,6	37,8	19,45	13,7	—	—	66,8
3	50,0	40,0	40,2	40,3	—	—	—	—	—
4	106,0	—	—	—	27,1	21,9	—	—	74,4
5	100,7	—	—	—	27,3	26,0	22,0	—	72,9
6	75,5	—	—	—	31,4	18,9	16,8	10,7	58,4
7	71,7	—	—	—	23,6	17,9	14,9	10,75	67,1
8	107,0	—	—	—	33,9	—	27,2	27,2	68,3

be explained by the view that the biochemical structures of the body tissues do not possess the ability to combine firmly with the preparation and they adsorb it for only a short time.

With the aim of verifing this hypothesis we carried out a series of experiments in vivo and in vitro. In the

experiments in vivo, the preparation was injected in a dose of 5 mg/kg intravenously, the animals were decapitated after 5 minutes or 2 hours and the radioactivity of the chemical fractions of the different organs determined.

As seen from Table 1, 5 minutes after injection the proteins and lipids contain comparatively little radioactive material. After 2 hours only traces of radioactivity remain in the proteins of the brain and the kidneys, but in the lipids of these organs the content of thioester falls noticeably. In the fractions of the liver no essential changes in the concentration of thioester take place during the two hours; in this period of time the concentration of thioester in the fractions of the lungs falls.

In the experiments in vitro we incubated a brain emulsion with different concentrations of the preparation (0.02-2 mg/g) for 2 minutes, after which the tissue was fractionated and the radioactivity of the fractions determined.

It can be seen from Table 2 that with a rise in the concentration of thioester in the homogenate the radioactivity of both fractions increases, and moreover there is a particularly well expressed direct relationship between the concentrations of thioester in the proteins and the amount of the preparation added to the tissue. In the lipid fraction the same relationship was expressed, although not to such a marked degree.

We employed still higher concentrations, but in no case were we able to reach saturation limits. Thus, no stechiometric relationships exist in the interaction of thioester with the chemical structures of the brain tissue.

In order to test the firmness of combination of the drug with the proteins of the brain, experiments were performed in which a brain emulsion was incubated for 30 minutes with radioactive thioester (0.2 mg/g) and then subjected to dialysis against cold tap water (thioester is readily soluble in water). From Table 3 it can be seen that after 24 hours the bulk of the radioactivity has passed off; during the next day the process of clearance proceeds more slowly.

The study of the distribution of radioactive thioester in the animal body has shown that the preparation does not form stable compounds with the biochemical structures of the brain tissue, and that its interaction with the tissues is effected not by the formation of covalent bonds, where stechiometric relationships are essential, but evidently by means of adsorption.

SUMMARY

The author studied the peculiarities of the distribution of thioester in the animal body in different ways of its administration in experiments in vivo and in vitro. It was demonstrated that no stechiometric relationship exists in the interaction of the thioester with the chemical structure of the brain tissue.

The study of the distribution of the radioactive thioester in the animal body demonstrated that there is no stability in retention of this preparation by the biochemical structures of the brain tissue. Its interaction with the tissue substrates takes place not by the formation of the covalent connections where stechiometric relationships are imperative, but evidently as a result of adsorption.

LITERATURE CITED

[1] A. M. Kats, Biull. Eksptl. Biol. i Med. 46, 10, 61-65 (1958).*

* Original Russian pagination. See C. B. Translation.

BROMINE METABOLISM IN THE THYROID GLAND OF GUINEA PIGS DURING EXPERIMENTAL HYPERTHYROIDISM AND IN RATS FED ON METHYLTHIOURACIL

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The study of the bromine metabolism of the thyroid gland in normal conditions and in various functional states is of considerable theoretical and practical interest. In this connection we carried out two series of experiments: in the first we studied the influence of experimental hyperthyroidism on the content of radiobromine in the thyroid gland of animals, and in the second, we studied the influence of methylthiouracil (MTU) on the appearance of injected bromine, labeled with Br^{82}, in the thyroid gland of animals. Further, in both series we studied the distribution of radiobromine between the protein and nonprotein fractions isolated from the tissue of the thyroid gland.

It was shown by a number of workers [1, 8, 9] that the bromine content of the thyroid gland is usually higher than in other tissues and, what is particularly important, higher than in the blood passing through the gland. As our own findings [2] showed, in thyrotoxicosis in man the bromine content of the thyroid gland rises considerably. On the other hand, E. N. Emel'ianova and Iu. B. Skebel'skaia [7] showed that during relatively brief administration of bromine to animals, hypertrophy and hyperplasia of the thyroid glands are observed.

1. The Influence of Experimental Hyperthyroidism on the Radiobromine Content of the Thyroid Gland in Guinea Pigs

The experimental method was as follows. Hyperthyroidism was produced in guinea pigs by injecting them with thyrotrophic hormone.* The hormone was dissolved in physiological saline and injected subcutaneously into the experimental guinea pigs for a period of five to seven days in a dose of 22.5-72.5 mg. The last dose of the hormone was injected simultaneously with a solution of sodium bromide, labeled with Br^{82}, 16-18 hours before decapitation of the experimental and control animals. The control animals were injected with radiobromine alone. Next the activity of the tissues was measured and the relative activity calculated (the activity of the blood was taken as 1). The results obtained are shown in Table 1.

From Table 1 it is seen that the experimental thyrotoxicosis in guinea pigs a considerable increase takes place in the weight of the thyroid gland. So far as the relative activity of the thyroid gland tissues is concerned, in hyperthyroidism it was considerably higher (1.79 ± 0.15) than in controls (1.32 ± 0.08). In other tissues which we investigated, no change in the relative activity of the bromine was observed in hyperthyroidism as compared with normal.

It was important to explain how experimental hyperthyroidism affects the appearance of Br^{82} in the protein fraction of the thyroid gland tissue. For this purpose the thyroid gland tissue was ground up in a mortar and the

* We take this opportunity to thank N. M. Rudenko for preparing the thyrotrophic hormone for us.

TABLE 1

Distribution of Bromides Labeled With Br^{82} in Certain Organs and Tissues of Guinea Pigs in Normal Conditions and in Experimental Hyperthyroidism

Number of animals investigated in four experiments	Control 12	Hyperthyroidism 12	Difference
Weight of thyroid glands in mg	35,6±1,61	73,8±2,22	38,2±2,74
Name of organs and tissues	Relative activity		
Blood	1	1	0
Thyroid gland	1,32±0,08	1,79±0,15	0,47±0,17
Pituitary	0,68±0,063	0,68±0,079	0,00±0,10
Nerves	0,66±0,026	0,67±0,038	0,01±0,05
Cerebral hemispheres	0,25±0,008	0,27±0,017	0,02±0,02
Midbrain	0,29±0,011	0,31±0,010	0,02±0,01
Cerebellum	0,29±0,012	0,31±0,012	0,02±0,01
Medulla	0,33±0,011	0,34±0,011	0,01±0,01
Spinal cord	0,38±0,038	0,36±0,017	0,02±0,04
Adrenals	0,34±0,01	0,41±0,05	0,07±0,05

TABLE 2

Content of Br^{82} in the Protein and Nonprotein Fractio of the Thyroid Gland Tissue of Guinea Pigs in Normal Conditions and in Hyperthyro sm

Experiment No.	Radiobromine injected (μCi/kg)	Bromine injected (mg/kg)	Material (thyroid glands)	Activity: nonprotein fractions (fissions, min)	Activity: %	Activity: protein fractions (fission/min.)	Activity: %	Total activity of 100 mg of tissue: fissions, min.	Total activity of 100 mg of tissue: %
1	2	3	4	5	6	7	8	9	10
38	16,36	2,4	Control	332	5,9	5280	94,1	5612	
				346	6,4	5020	93,6	5366	
			Average		6,15			5490	100
			Hyperthyroidism (thyrotropic hormone injection)	500	7,4	6840	92,6	7340	
				355	5,1	6650	94,9	7005	
				342	5,7	5730	94,3	6088	
			Average		6,07			6811	124
38 a	16,36	2,4	Hyperthyroidism (thyrotropic hormone + + NaBr injected)	255	4,0	6180	96,0	6435	
				145	2,5	5640	97,5	5785	
			Average		3,25			6110	111
37	12,2	1,86	Control (glands from 3 guinea pigs)	532	19,9	2132	80,1	2664	100
			Hyperthyroidism (thyrotropic hormone injected) (glands from 3 guinea pigs)	382	12,3	2713	87,7	3095	116

TABLE 3

The Influence of Methylthiouracil on the Appearance of Bromine (Br^{82}) and Iodine (I^{131}) in the Thyroid Gland of Rats

Experiment No.	№ 10* 30/I—8/II 1952		№ 20* 4/III—14/III 1952		№ 67$_{1,2}$ 14/VI—24/VI 1955			№ 85 24/XII—2/I 1956		№ 87 10/I—1/II 1956		№ 76 17/IX—1/X 1955	
Br^{82} injected (μC/kg)	14,7		3,45		16,36			19,6		30		0,1 μC/kg I^{131}	
Bromine injection (mg/kg)	0,54		0,46		0,54			3,0		6,16		0 (without vehicle)	
Duration of experiment (in days)	10		10		10			10		20		10	
Number of animals (n)	Control 10	Experiment 10	Control 10	Experiment 5	Control 5	Experiment 5	Control 6	Experiment 6	Control 6	Experiment 6	Control 5	Experiment 3	Control 3
MTU given (mg)	0	300	0	300	0	30	300	0	300	0	600	0	300
Weight of thyroid glands (in mg)	16,6±1,4	61,3±3,6	15,2±1,0	49,0±4,1	13,8±1,4	39,4±4,8	48,4±7,7	21,8±1,6	59,3±2,4	24,5±1,5	98,2±4,6	13,7±1,1	56,0±6,1
As a % of control		359		327		278	343		268		392		400
Relative activity of the thyroid glands												Activity of 100 mg of thyroid gland tissue (in fissions, min)	
As a % of control	1,21±0,08	1,75±0,19	1,51±0,10	1,62±0,16	1,12±0,12	1,66±0,031	1,57±0,15	0,88±0,05	1,26±0,06	0,86±0,04	1,95±0,15	69260±8730	4130±420
		145		108		148	140		143		227		5,9

*Prof. I. A. Eskin and E. B. Pavlova took part in experiments Nos. 10 and 20.

proteins were precipitated with 10% trichloroacetic acid in the cold. The protein precipitate was next washed 4-5 times with trichloroacetic acid with the addition of inactive sodium bromide, after which it was dissolved in weak alkali. The solutions containing the protein and nonprotein fractions were transferred to disks and evaporated to dryness and their activity was measured. The results obtained, calculated per 100 mg dry gland tissue, are shown in Table 2.

It may be seen from Table 2 that the total bromine content of the thyroid gland in guinea pigs is increased in hyperthyroidism as compared with normal (columns 9 and 10). The preliminary administration of bromine slightly reduces this effect. It may also be seen (column 6) that in hyperthyroidism there is a tendency towards reduction of the quantity of radiobromine entering the protein fraction of the thyroid gland tissue. As was to be expected, the preliminary administration of bromine considerably reduces the quantity of radiobromine entering the protein fraction of the thyroid gland tissue as compared with normal.

2. The Influence of Methylthiouracil on the Appearance of Radiobromine in the Thyroid Gland

The experimental method was as follows. Experimental rats received methylthiouracil in addition to their diet for a period of 10-20 days. The total amount of methylthiouracil taken by each animal differed in the different experiments – 30, 300 and 600 mg. Later on both experimental and control animals were given subcutaneous injections of sodium bromide solution, labeled with Br^{82}, after which, 15-18 hours later, they were decapitated. Next the activity of the blood and the thyroid glands was measured and, taking the activity of the blood as unity, the relative activity of the thyroid gland tissue was calculated. The conditions for the performance of the experiments, as well as the results obtained, are shown in Table 3.

It may be seen from Table 3 that the administration of MTU causes a considerable increase in the weight of the thyroid glands compared with controls. It may also be seen that in five of the six experiments a statistically significant increase was observed in the activity of the thyroid gland tissue of the experimental animals as compared with the controls, which proves the accumulation of Br^{82} in this tissue. It should be pointed out that the largest accumulation of Br^{82} was observed in experiment No. 87, i.e. after the largest dose of MTU.

TABLE 4

Influence of Methylthiouracil (MTU) on the Content of Br^{82} and I^{131} in the Protein and Nonprotein Fractions of the Thyroid Gland Tissue of Rats

Experiment No.	Thyroid gland tissue	Number of animals	MTU administered (in mg)	Br^{82} injected (in μC/kg)	I^{131} injected (in mg/kg)	Bromine injected (in mg/kg)	Iodine injected (in mg/kg)	Activity of the protein fraction: fissions, min	Activity of the protein fraction: in %	Activity of the nonprotein fraction: fissions, min	Activity of the nonprotein fraction: in %	Total activity in fissions, min
1	2	3	4	5	6	7	8	9	10	11	12	13
85	Control	6	0	19,62	—	3	—	264	7,6	3 190	92,4	3 454
	Experiment	6	300	19,62	—	3	—	83	1,8	4 650	98,2	4 733
87	Control	6	0	30,6	—	2	—	45	1,2	3 659	98,8	3 704
	Experiment	5	600	30,6	—	2	—	77	0,6	11 863	99,4	11 940
76	Control	3	0	—	0,39	—	0	22 311	93,1	1 654	6,9	23 965
	Experiment	3	300	—	0,39	—	0	2 658	82,6	558	17,4	3 216

Although the action of MTU on the iodine metabolism of the thyroid gland has often been investigated [4, 5, 6], we carried out experiments with radioiodine in order to compare the action of MTU on the appearance of bromine (Br^{82}) and iodine (I^{131}) in the thyroid gland under identical conditions. The results of this experiment (No. 76) are shown at the end of Table 3. It may be seen from these that the administration of MTU causes a very considerable fall in the content of radioiodine in the thyroid gland – down to 5.9% as compared with the normal value of 100.

By comparing the results obtained with bromine and iodine (see the bottom line in Table 3), it may be seen that the influence of MTU on the content of bromine and iodine in the thyroid gland (after an identical interval of time) is opposite – the content of Br^{82} is increased 1.5-2.0 times, whereas the content of I^{131} is sharply reduced – by 17 (!) times.

We also studied the influence of MTU on the distribution of bromine between the protein and nonprotein fractions of the thyroid gland tissue. For this purpose the thyroid gland tissues of experimental and control rats containing Br^{82} (experiments Nos. 85 and 87), after measurement of their activity, were scraped from the glass disk and fractionated. The same course was adopted with the thyroid gland tissues of rats receiving I^{131} (experiment No. 76). Fractionation was done by the method described above, with the exception that the weighted samples were taken from air-dried rather than fresh gland tissue. The results obtained on the activity of the fractions, expressed as the number of fissions per minute and as a percentage, are shown in Table 4.

From Table 4 (column 10) it may be seen that in experimental rats taking MTU in the diet, the percentage content of radiobromine in the protein fraction of the thryroid gland tissues, never very high, is still further reduced (1.8% instead of 7.6% and 0.6% instead of 1.2%). As may be seen from column 13, the total content of radiobromine in the thyroid gland of rats receiving MTU in the diet increases. It may be seen that the percentage content of radioiodine in the protein fraction of the thyroid gland tissue of the experimental animals is also reduced (82.6% instead of 93.1%). It should be particularly emphasized that the absolute radioiodine content of the thyroid gland tissue of the experimental rats is sharply reduced – in samples of equal weight an activity of 3216 fissions/ min instead of 23,965 fissions/ min is observed.

SUMMARY

A significant increase in the weight of the thyroid glands and of the bromine content was noted in experimental hyperthyroidism of ginea pigs. The relative quantity of the protein-combined bromine does not change. Preliminary administration of bromine greatly decreases the entrance of bromine into the protein fraction of the thyroid gland tissue.

The percentage of bromine in the protein fraction of the thyroid gland tissue is considerably diminished in rats fed methylthiouracil, whereas the total amount of bromine entering the thyroid gland of experimental animals rises.

LITERATURE CITED

[1] I. N. Verkhovskaia, Izv. Akad. Nauk. SSSR, Ser. Biol. 1, 114-127 (1950).

[2] I. N. Verkhovskaia and L. M. Tsofina, Biull. Eksptl. Biol. i Med. 35, 4, 47-49 (1953).

[3] E. N. Emel'ianova, Biull. Eksptl. Biol. i Med. 11, 3, 274-277 (1941).

[4] M. T. Zaks, Uspekhi Sovremennoi Biol. 23, 37-46 (1947).

[5] Ia. M. Kabak, Uspekhi Sovremennoi Biol. 28, 187-210 (1949).

[6] Ia. M. Kabak and A. Freidman, Doklady Akad. Nauk SSSR 51, 9, 735-738 (1946).

[7] Iu. B. Skebel'skaia, Doklady Akad. Nauk SSSR 94, 1, 165-168 (1954).

[8] E. J. Baumann, N. Z. Searle , A. A. Yalow, et al., Am. J. Physiol. 1956, v. 185, p. 71-76.

[9] I. Perlman, M. E. Morton and I. L. Chaikoff, Am. J. Physiol. 1941, v. 134, p. 107-113.

MORPHOLOGY AND IMMUNOLOGY

PENETRATION OF THE SKIN BY MICROORGANISMS

COMMUNICATION I. EXPERIMENTAL DATA ON THE PASSAGE OF MICROORGANISMS THROUGH SEMIPERMEABLE MEMBRANES

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The ability of microorganisms to pass through the skin, and the conditions for this have interested research workers for a long time. According to reports in the literature the passage of microorganisms through the skin is limited by processes of self-cleansing taking place in that tissue; it is also connected with the whole complex of protective properties of a biological nature which proceed inside the skin.

The variety of conditions limiting the passage of microorganisms through the skin led us to undertake the solution of this problem in stages.

The majority of authorities [1, 3, 4, 8 and others] associate the penetration of the skin by microorganisms in man with some form of trauma to the epithelium – cuts, scratches or other injuries. A far smaller number of workers assert that the outer layer of the human skin, if undamaged, does not present a sure defense against the organisms causing disease [5-7].

We undertook to investigate in the first place the part played by the epidermis in the process of entry of various microorganisms through the skin.

We obtained the epidermis from cadaver skin by the method of A. V. Pshenichnov and B. I. Raikher [2]. At predetermined areas of the skin burns were produced by means of hot compresses. The epidermis formed a blister and was streched over a specially prepared glass cannula with a wide portion, and the narrow portion of the cannula was passed through a cork stopper which closed a flask in such a way that the lower edge of the membrane was in contact with the nutrient medium in the flask. Through the narrow end of the cannula was injected a 24-hour culture of Vibrio metchnikovii in peptone water, in a volume of 0.5 ml; every day thereafter for the next 3-5 days a microbiological examination was made of the peptone water contained in the flask.

The results differed in accordance with the area of the skin from which the epidermomembrane was obtained. Where the epidermis was taken from the upper third of the skin of the leg, then all the cultures in peptone water and the subcultures on alkaline agar showed the presence of the vibrio; it can be assumed that here a part was played by the porosity of the membrane resulting from the presence of hairs on the skin of the leg. On the other hand, the epidermomembrane from skin of the posterolateral area of the abdomen, devoid of hairs, was impermeable to the vibrio.

However, dead epidermis of human skin, with its biological properties modified by the high temperature, could not give results of a reliable physiological order. For this reason we subsequently used the skin of a living frog (in spring) for the investigations.

Each frog was decapitated immediately before the experiment, and the skin of the trunk removed aseptically. Pieces of skin of the required dimensions were cut out and stretched over the wide portion of the glass cannula,

which was then introduced into the flask of peptone water. Depending on the aim of the experiment the skin was stretched with either its epithelial or its serous surface uppermost. A 24 hour culture of the microorganism was introduced inside the glass tube.

All the membranes of frogs' skin, stretched with the epithelial and serous sides uppermost, and a control cellophane membrane were impermeable to V. metchnikovii in observations lasting 8 days.

Control experiments with a membrane of 3 layers of gauze showed that in the conditions appertaining, the vibrios which passed into the flask of peptone water were capable of surviving in it for 2-3 days or more.

Later experiments with Bacillus mirabilis, using suitable nutrient media for this organism, showed that if placed on the epithelial surface of the frog's skin, it will pass through it in under 24 hours. If B. mirabilis is placed on the serous surface of the skin, it could be found in the nutrient medium only after daily observation for 16 days. However, this period of time cannot serve as proof of passage of B. mirabilis through the skin of the frog, for after 16 days processes of autolysis might have begun to occur in the skin as a result of disturbance of its physiological requirements. In control experiments with a cellophane membrane, B. mirabilis was found in the nutrient medium beyond the membrane after 48 hours.

A further control was a 1% solution of trypan blue. This solution was poured on to the epithelial surface of the skin and the cellophane membrane, which were then placed in flasks containing petone water and tap water. In neither liquid was the dye found after 8 days of observation.

Thus, living frogs' skin and cellophane were both impermeable to V. metchnikovii and trypan blue. Only B. mirabilis was able to surmount both obstacles – dead or living – and in the latter case, in the direction from the epithelial to the serous surface.

The numerous epidemiological observations indicating the possiblity of leptospiral infection through the skin, and consequently a passage of leptospires through it, led us to carry out a series of experiments by the method already described, with leptospires of 2 strains – BShch and Dubna 4. As a nutrient medium for the leptospires we used the ordinary Vervoort-Wolfe medium prepared with the addition of buffer solutions. In 50% of the experiments the skin was stretched with its epithelial side uppermost and in the same number, with the serous surface uppermost.

The experiment with leptospires was carried out only if not less then 10-15 specimens were found in a field of vision in the culture.

Inspection of the nutrient medium for the presence of leptospires was carried out with dark-ground illumination 2 hours after introduction of the culture and daily thereafter for 18 days. The presence of living, motile leptospires was recorded.

The results of experiments with the skin of winter frogs showed that leptospires could pass through it when its epithelial surface was uppermost; this was seen in the flask after only 2 hours, in a number of one in 2-3 fields of vision (see Table).

Leptospires did not pass through the skin of these frogs turned with the serous side uppermost, nor through the cellophane membrane. However, leptospires were found in a number of one in 2 fields of vision at the other side of a control membrane of 3 layers of gauze.

Leptospires did not pass through the skin membranes of summer and spring frogs with the epithelial or serous side uppermost; they could begin to appear in the nutrient medium (when nonmotile) on the 11th-18th day as a result of breaking of the membrane by the onset of autolysis.

It follows from the foregoing that the living skin of summer and spring frogs is impermeable to leptospires both from the epithelial to the serous layer and vice versa. The skin of winter frogs was found to be permeable to leptospires in the direction from the epithelial to the serous surface.

In later experiments by the same method we tested the permeability of the skin of summer and spring frogs to the following microorganisms: 1) Staphylococcus aureus; 2) Staphylococcus citreus; 3) Bacillus anthracoides and 4) Escherichia coli. Permeation or absence of microorganisms was checked by daily cultures for 5-7 days on suitable elective and differential media for each of the above microorganisms. The experimental results showed that not all the microorganisms pass through the skin of the living frogs to an equal degree. This unequal permeation

Permeability of the Membrane From the Skin of the Frog to Leptospires and Microorganisms

Membranes	Leptospires: time of observation							Microorganisms: time of observation – from 1 to 7 days			
	2 hr	1 days	2 days	1–10 days	11 days (rupture of mem.)	1–17 days	18days (rupture of mem.)	name of microorganisms: St. aureus	St. citreus	E. coli	B. anthracoides
	winter frogs			summer frogs							
1	2	3	4	5	6	7	8	9	10	11	12
Skin of the frog from the outer surface	1/2 1/3	1/2 0	0 0	0 0	many/1 many/1	0 0	many/1 many/1	55,5	16,6	16,6	100
Skin of the frog from the inner surface	0 0	0 0	0 0	0 0	many/1 many/1	0 0	0 0	0	22,4	5,5	5,5
Cellophane	0	0	0	0	0	0	0	0	33,3	0	0
Gauze	1/2	1/2	1/2	many/1	many/1	many/1	many/1	100	100	100	100

Notes. 1) $\frac{1}{2}$, $\frac{1}{3}$ – one per 2-3 fields of vision, many/1 – many in one field of vision. 2) In columns 9-12 the intensity of permeation is expressed in percent.

of microorganisms through the skin depends primarily on its position, i.e. whether it has its epithelial or its serous suface uppermost. There is no doubt that the degree of permeation of microorganisms through the skin is also connected with the distinctive features of the microorganisms themselves. Nevertheless the same microorganisms passed from the epithelial to the serous surface much more easily than in the opposite direction.

It is seen from the Table that the positive results during permeation of microorganisms from the epithelial to the serous surface varied from 16.6 to 100%. The greatest ability to pass through the skin in this direction was shown by B. anthracoides (100% permeation), next followed Staph. aureus (55.5% positive results). Staph. citreus and E. coli each had equal ability to pass through the skin. Passage of microorganisms from the serous to the epithelial surface took place with much greater difficulty, and the number of positive results did not exceed 22.4%. The greatest fall in the intensity of permeation in this direction was shown by B. anthracoides and Staph. aureus.

This difference in the power of permeation cannot be attributed to the difference in the size of the microorganisms (the smallest and largest microorganisms pass with equal ease). Neither can this phenomenon be explained by the presence or absence of motility in the microorganisms or by their different resistance to physical and chemical agents.

The factors facilitating the permeation of the skin by B. mirabilis, B. anthracoides, Staph. aureus and leptospires may be extremely diverse, and their role in this process requires further study.

The control gauze membrane was found to be permeable to all the microorganisms used in the work: Staph.

aureaus and citreus, B. anthracoides, E. coli, leptospires and V. metchnikovii. Cellophane allows the passage of B. mirabilis and Staph. citreus only; the remaining microorganisms (E. coli, B. anthracoides, Staph. aureus and leptospires) did not pass through cellophane under the above experimental conditions.

SUMMARY

The ability of epidermis to serve as a barrier to microbes is preserved to a certain extent on the epidermomembranes prepared by Fshenichnov's method from the skin of human cadaver.

The barrier function of live uninjured frog's skin varies with respect to different microbes. Thus, B. anthracoides, Staphylococcus areus, B. prodigiosus and leptospires penetrate the frog's skin with comparative ease in the direction from the epithelial surface to the serous one. Penetration of microorganisms in the opposite direction either does not take place at all or is more difficult.

LITERATURE CITED

[1] A. A. Imshenetskii, Zhur. Microbiol. i Immunobiol. 7, 2, 159-163 (1930).

[2] A. V. Pshenichnov, B. I. Raikher and E. T. Isakova, Zhur. Mikrobiol., Epidemiol. i Immunobiol. 10-11, 54-59 (1945).

[3] S. M. Frid, Collected Papers on the 25th Anniversary of the Belorussian State Medical Institute, pp. 163--207, Minsk (1939).*

[4] P. V. Tsiklinskaia, in the book: Studies on Microorganisms, by S. I. Zlatogorov, pp. 202-254 (1917).*

[5] G. V. Epshtein, Pathogenic Protozoa, Spirochetes and Fungi, Moscow-Leningrad (1931).*

[6] Babes, Compt. rend. Soc. biol. 1883, Cited by Frid (1939).

[7] Garre, Fortschritte der Medicin, t. III, 1885, Cited by Nikol'skii, 1893 and by Frid, 1939.

[8] Gessner, Microscopische Untersuchungen ueber den Bakteriengehalt der normalen menschlichen Haut. Diss. 1890, Cited by Nikol'skii, 1893.

* In Russian.

EXPERIMENTAL BIOLOGY

THE ACTION OF A CHEMICAL IRRITANT (OZOKERITE) ON THE EPIDERMIS AFTER LOCAL X-RAY IRRADIATION

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During the histological study of the reaction of the skin to x-ray irradiation, the classic picture of the changes in the epidermis of the guinea pig was decribed by Miescher [12], who found the development of polymorphism of the nuclei, the appearance of multinuclear giant cells and degeneration of the cells of the basal layers. Similar changes were also described in the epidermis of the rabbit [1, 4, 6 and others]. Under these circumstances it must be pointed out that in morphological investigations it is customary to use large doses of x-rays: 1800, 3600, 4000 and 10,000 r and over, and the biopsy is taken several weeks or months after irradiation. So far as changes in the epidermis are concerned, after small doses and shorter periods of observation, much less material is available for the morphologist. Miescher [12], for instance, points out that after a dose of 800 r the histological picture of the epidermis of the guinea pig generally speaking remains normal, although isolated multinuclear cells are encountered and disturbances occur in the course of karyokinesis. L. V. Funshtein [10], using rabbits, which are less sensitive to irradiation than guinea pigs, could find no definite changes in the epidermis with a dose of 466 r.

In our investigation we studied the changes in the epidermis resulting from irradiation with x-rays in doses of 500 and 1000 r , and from stimulation of the physiological regeneration after such irradiation. As a stimulator of the processes of regeneration we used an ozokerite paste, whose action on regeneration of the skin we have often studied in various experimental conditions [7, 8].

EXPERIMENTAL METHOD

Experiments were carried out on 30 adult rabbits (weighing about 2 kg). The experiment began with irradiation of the right ear of the rabbit with x-rays in a dose of 500 r (first series) or 1000 r (second series). Conditions or irradiation: voltage 160 kv, current 5 mA, focal distance – 30 cm, dose rate 70 r/ min. In one group of animals of each series biopsy specimens were taken from symmetrical areas of both ears 2 days after irradiation, and in the other group – after 2 weeks (14 days). After removal of the biopsy, the inner surface of both ears was smeared with oxokerite paste once daily. The next biospy specimens were taken from all the animals 4 days after the first application of paste; in addition in some cases additional biopsies were taken 8, 16, 30 and 60 days after the first application of paste. The specimens were fixed in 20% formalin, and frozen sections were cut and stained with hematoxylin-eosin.

During microscopic study of the preparations, by means of an ocular micrometer the total thickness of the stratum germinativum and the stratum granulosum of the epidermis of the inner surface of the ear was measured, and the rows of cells in these layers were counted. The result of the counting and measurement was expressed as a formula in which the first item represents the number of rows of cells in the stratum germinativum, the second the number of rows of cells in the stratum granulosum (if the stratum granulosum is not continuous the figure 1 is placed in brackets), and the sum represents the total thickness of the epidermis (without the stratum kerativum in microns. This enables the reaction of the epidermis to be expressed quantitatively and the changes in reactivity to be shown in cases where there are no qualitative morphological changes in the cutaneous epithelium.

EXPERIMENTAL RESULTS

First series of experiments. There were 15 animals in this series. In 5 rabbits the first biopsies were taken 2 days after irradiation, and in 10 rabbits after 2 weeks. In the unirradiated ear the condition of the epidermis of the inner surface was expressed by the formula 2-3 + (1) = 20 μ. This means that in the stratum germinativum there were 2 or 3 rows of cells, and in the stratum granulosum one incomplete row of cells, while the thickness of the epidermis without the stratum kerativum was 20 μ. At the same time the average result for the irradiated ears when the biopsies were taken after 2 days was 3-4 + 1 = 26 μ, and after 2 weeks, 3 + 1 = 24 μ. These figures indicate that as a result of irradiation the epidermis became thicker, and also that the thickening of the epithelium was accompanied by an increase in the number of cells in that tissue.

A characteristic feature of the epidermis of the irradiated ears is the increased dimensions of the nuclei in certain cells. We saw nuclei enlarged to twice their normal size 2 weeks after irradiation in 7 of the 10 animals. However 2 days after irradiation these nuclei were present in only one of the 5 rabbits investigated.

The action of ozokerite on the epidermis of the irradiated and unirradiated ears gave different results. If the smearing with ozokerite began 2 days after irradiation the state of the epidermis after four applications was expressed in the unirradiated ear by the following formula: 6 + 1-2 = 42 μ, and for the irradiated ear: 4-5 + 1 = = 34 μ; in the group of animals in which the smearing with ozokerite began 2 weeks after irradiation, the epidermis of the unirradiated ear corresponded to the formula 5-6 + 1 = 43 μ, and of the irradiated ear, 4-5 + 1 = = 38 μ. These figures show that preliminary irradiation of the ear with a dose of 500 r lowers the reaction to subsequent stimulation; in fact the lowering of the reaction is still greater if the original state of the epidermis before the first application of the paste is considered. The increase in thickness of the epidermis of the irradiated ear in cases where application of ozokerite began 2 days after irradiation was 2.8 times smaller than that of the unirradiated ear; meanwhile where application of paste began 2 weeks after irradiation, this difference was reduced and the increase in the thickness of the irradiated epidermis was only 1.8 times smaller than the increase in the thickness of the epidermis of the unirradiated ear.

Smearing the skin with ozokerite leads to the disappearance of the enlarged nuclei from the epidermis, i.e. to the restoration of the normal structure of its cells. When the application of the paste began 2 days after irradiation, enlarged nuclei were not found after 4 days nor when the biopsy was taken at later periods. Where the application of ozokerite began 2 weeks after irradiation, enlarged nuclei were present in the epidermis of 3 animals after 4 daily applications, but were not encountered later.

When the ozokerite was applied for longer periods of time (8, 16, 30 and 60 days), we found that the fall in reactivity of the irradiated epidermis was a temporary condition. Only 16 days after the onset of application of paste not one case of asymmetry could be found; the reaction of the epidermis was the same in the irradiated and unirradiated ears. The same thing was observed also at later periods.

Second series of experiments. This series comprised 15 animals also. In 5 rabbits the first biopsies were taken 2 days after irradiation, and in 10, after 2 weeks. In contrast to the first series the dose of x-rays was 1000 r. The experimental method was as before.

Comparison of the state of the epidermis of the irradiated and unirradiated ears showed that as a result of irradiation with a dose of 1000 r, the epidermis became thicker than that of the unirradiated ear in 12 of the 15 animals; in the other three cases no difference was found. Under these circumstances, 2 days after irradiation the epidermis corresponded to the formula 3 + 1 = 24 μ, and 2 weeks afterwards, 3 + 1 = 22 μ. Histological study of the preparations showed the presence of cells with enlarged nuclei in the epidermis of the irradiated ear in 3 of the 10 animals in which biopsies were taken 2 weeks after irradiation.

The rabbits of this series showed a lowering of reactivity towards both x-rays and ozokerite. Furthermore the reaction of the epidermis to the application of ozokerite to the irradiated ear was significantly weaker than to the unirradiated ear. In the group of animals in which the application of paste began 2 days after irradiation, the epidermis of the unirradiated ear corresponded at first to the formula 3 + (1) = 20 μ, and after four applications, 3-4 + 1 = 29 μ; the epidermis of the irradiated ear was respectively 3-4 + 1 = 24 μ and 3-4 + 1 = 26 μ. Consequently, as a result of four applications of ozokerite the thickness of the epidermis of the unirradiated ear increased by 45% and that of the irradiated ear by only 8%, i.e. the increase was almost 6 times smaller.

In the group of animals in which the application of paste began 2 weeks after irradiation, the reaction was

expressed rather more strongly, but here also the increase in the thickness of the epidermis of the irradiated ear as a result of four applications of ozokerite was 3 times smaller than on the unirradiated side (32% against 100%). The epidermis of the unirradiated ear at first corresponded to the formula 2-3 + (1) = 18 μ, and after application of the paste it took the form 4 + 1 = 36 μ; on the irradiated ear, before application of paste the epidermis corresponded to the formula 3 + 1 = 22 μ, and after application, 3-4 + 1 = 29 μ.

As a result of 4 daily applications of ozokerite, in some animals the number of enlarged nuclei in the epidermis was increased, or where such nuclei were not seen before the applications were started, they began to appear. After application of ozokerite for 16 days, polymorphism of the nuclei of the epidermis was not yet found, and the reaction to ozokerite was expressed equally in the irradiated and unirradiated ears.

Our results show that the action of x-rays in doses of 500 and 1000 r on the epidermis of the rabbit has a dual character. On the one hand, after irradiation of the epidermis it thickens and there is an increase in the number of cells in its stratum germinativum and stratum granulosum; this earlier x-ray reaction may be regarded as stimulation of physiological regeneration on its part. On the other hand the irradiated epidermis, when acted upon by ozokerite, is found to be less stimulated into proliferation than is the unirradiated; this demonstrates the presence of an inhibitory, retarding action of x-ray irradiation on the course of the regenerative processes in the epidermis. These findings are in agreement with those of A. A. Zavarzin and G. S. Strelin [5], who in another animal and by the use of a different method, concluded that x-ray irradiation has a dual action.

According to our findings, the inhibitory action of x-rays in a dose of 500 r is less pronounced than in a dose of 1000 r. This is in accordance with the generally accepted view that the reaction depends on the size of dose applied, and no special discussion is called for.

Comparison of our results of experiments carried out 2 days and 2 weeks after irradiation shows that the inhibitory action of x-ray irradiation on the epidermis is more marked in the first few days than after 2 weeks.

In G. S. Strelin's laboratory, where the reaction of the corneal epithelium to x-ray irradiation was determined by the mitotic activity, I. B. Bychkovskaia [3] obtained results which showed that after small doses (2-50 r) the suppression of mitotic activity continues for a few hours in all and it is then restored. Workers using irradiation in large doses [2, 9] point out that the regenerative power of the tissues is restored a few months after irradiation. For this reason we consider that our results, obtained with average doses and by the use of a rather different method, are in agreement with these reports in the literature. At the same time we found that the reactivity of the tissues during the latent period is already modified, and this modification can be demonstrated by the application of an additional stimulus, namely ozokerite.

One of the signs of the action of radiation on the epidermis is enlargement of the cell nuclei, which was also observed in our material. At a dose of 500 r, enlargement of nuclei was observed in one case after only 2 days, and in the majority of cases 2 weeks after irradiation; four applications of ozokerite led to the disappearance of the large nuclei from the epidermis. At a dose of 1000 r, enlarged nuclei appeared only 2 weeks after irradiation, and then in only a minority of the animals, but they were most often found 4 days after the start of application of ozokerite; at later periods after the start of the applications (8 or 16 days or over) large nuclei did not appear. These findings show that ozokerite is similar in its action on the irradiated epidermis to yellow paraffin [2] and naphthalanum [11], and brings about very rapid restoration of the normal structure of the epidermis.

SUMMARY

X-ray irradiation of a rabbit's ear with the dose of 500 and 1000 r results in thickening of the epidermis and in increase of the number of cellular layers. In additional irritation with ozokerite the irradiated epidermis gives a weaker reaction than the one which was not subjected to irradiation. X-ray irradiation produces a double effect: at first it intensifies the physiological regeneration of the epidermis, and later it decreases the reactivity of this layer of the skin.

LITERATURE CITED

[1] A. A. Braun and G. N. Orlova, Doklady Akad. Nauk SSSR. 73, 4, 849-852 (1950).

[2] Idem, Voprosy. Eksptl. Biol. i Med. 2, 208-211 (1952).

[3] I. B. Bychkovskaia, Biull. Ekspti. Biol. i Med. 34, 10, 53-56 (1952).

[4] V. G. Garshin and M. A. Zakhar'evskaia, Arkh. Patol. 2, 72-75 (1953).

[5] A. A. Zavarzin and G. S. Strelin, Vestnik Rentgenol. i Radiol. 6, 183-205 (1928).

[6] B. I. Il'in, Clinical and Morphological Changes in the Skin, Candidate Disseration, Leningrad (1956).*

[7] I. F. Prizhivoit, Biull. Ekspti. Biol. i Med. 12, 45-48 (1952).

[8] Idem, Ibid.. 7, 57-61 (1953).

[9] B. Tokin, Doklday Akad. Nauk SSSR, 35, 7, 245-248 (1942).

[10] L. V. Funshtein, The Therapeutic Use of Radioactive Cobalt, pp. 41-54, Moscow (1956).*

[11] Idem, Med. Radiol. 6, 31-35 (1956).

[12] G. Miescher, Strahlentherapie, 1938, Bd. 61, S. 4-38.

* In Russain.

PRELIMINARY CONSERVATION OF THE GONARDS OF BIRDS BEFORE TRANSPLANTATION INTO A RECIPIENT

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One of the main causes of failure to transplantates to take is known to be delay in their vascularization, leading to tissue necrosis in the grafted organ. In order to overcome this during transplantation of gonads in birds it is almost impossible to utilize such surgical procedures as anastomosis of vessels (because of the small size of the material) or one-stage parabiosis (on account of the deep situation of these organs within the cavity).

In this connection it is essential to devise biological methods which would stimulate cell division in the tissues of the graft and the bed, or would bring about the maintenance of an isotonic state of the transplanted glands until a blood supply was established.

EXPERIMENTAL METHOD

The effectiveness of biological stimulation of transplantates, according to V. P. Filatov, has been widely tested on a variety of objects. When making use of this method of treatment of transplanted gonads in birds, we slightly modified it, and as the medium in which the grafts were preserved we selected allantoic and amniotic fluids from embryos.

The choice of allantoid embryonic fluid was made because it contains products of protein decomposition such as ammonia, urea and allantoin, which are used for stimulation of regenerative processes in wounds. In this particular fluid the components listed are present in association with each other, and it might be expected that without requiring any special preparation, this sterile fluid would be effective as a general stimulator. Further, as shown by research in B. P. Tokin's laboratory, the allantoic fluid of the embryo posseses bactericidal properties.

In other series of experiments we treated the grafts with amniotic fluid from embryos in order to maintain the transplanted gonads in an isotonic state until the establishment of a blood supply. The amniotic fluid of the embryo, being a natural isotonic medium adapted for the existence and development of the young growing tissues of the embryo, might promote the existence and growth of the regenerating tissues of the graft; in addition the bactericidal properties of the amniotic fluid of the embryo were demonstrated in B. P. Tokin's laboratory in birds.

Some of the grafts were preserved in blood serum and in the fluid which bathes the lens of the eye, or simply kept in cold storage without the use of any of these biological media.

For the experiments we used fowls of the Leghorn, Australorp and New Hampshire breeds. Homoplastic transplantation was carried out on them. The recipient birds were subjected to bilateral castration, after which one gonad of the same sex was implanted. Both male and female gonads were transplanted.

Most of the birds underwent the operation at the age of one month. In isolated cases their ages varied from 15 days to 2 ½ months, but the comparable series of experiments were set up to provide analogy in this and other features (sex, breed).

The gonads were extracted through an incision between the two last ribs, by means of iris forceps, and were transplanted to the recipient through a corresponding incision, into the depression between the lung and adrenal,

so that survival of the graft could be distinguished from regeneration of the recipient's own gonads. In this particular bed the grafts could be fixed without suturing (which interferes with the survival of small grafts), since in this situation the immobility of the transplanted gonads is not disturbed by peristalsis in the internal organs of the recipient.

For the preliminary treatment of the grafts with the fluids mentioned above, we used the following method.

Treatment of the grafts with allantoic and amniotic fluids. The hatched egg, as soon as it was taken from the brood-hen, was disinfected with alcohol. Using sharp scissors with fine, pointed ends, a small piece of the shell was cut away from the egg, near the obtuse end. At the same time an opening was made in the outer shell membrane, after which the allantois settled to the bottom. With a sterile needle the allantoic membrane was perforated; through the orifice thus formed the allantoic fluid was poured into a sterile tube and kept in the refrigerator at a temperature of +3°C. After this the amnion was pierced and the amniotic fluid poured into another sterile tube which also was placed in a refrigerator at a temperature of +3°C. The grafts were immersed in these tubes of fluid and stored in the refrigerator for 2-3 days.

For the purpose of obtaining these fluids we used 17-19 day embryos, for at this period the antibiotic properties of the amniotic fluid increase because of the using up of the albuminous membrane.

Treatment of the grafts with blood serum. Serum was prepared in the usual manner. Fresh blood was collected in a clean, sterile tube, allowed to stand for a few minutes and then centrifuged. The serum was kept in a refrigerator at a temperature of +3°C. The grafts were kept in the serum until the moment of grafting to the recipient (for 2-3 days).

Treatment of the grafts with the fluid bathing the lens of the eye. The eyes were removed from a fowl dying at operation and placed in clean tubes in the refrigerator at +3°C. Grafts were inserted through a small incision in the membranes of these eyes in the region of the pupil and kept there for about 3 days.

In some cases the grafts were preserved in the cold without treatment with any of these fluids mentioned and were placed directly in a sterile tube in the refrigerator at a temperature of +3°C.

TABLE 1

Survival Rate of Gonads not Subjected to Preliminary Treatment

Total number of experiments	Number of experiments with indeterminate results		Number of experiments in which absorption of the grafts took place	Number of survivals	
	result not known	result indefinite		Number of cases	%
36	2	3	27	4	12.9

Survival of the grafts was judged on the basis of such visual findings as the firm adherence of the graft to the bed, the absence of exudation and of encapsulation of the transplanted organ, and also by the healthy outward appearance of the graft (normal color, normal or increased size).

When interpreting the results, we took into considration not only prolonged and stable survivals but also primary "takes," in which the graft was fixed for a short time after transplantation, since both were equally exposed to the influence of the treatment of the grafts on their condition in the period immediately after grafting. (Subsequent absorption of already surviving grafts is largely due to immune relationships between donor and recipient).

Operations were performed on 152 birds.

The place at which the experiments were carried out and the birds kept was the premises of the Department of Darwinism and Genetics of the Kiev State University and the "Severinovka" collective farm in the Makarov district of Kiev region.

TABLE 2

Survival Rate of Gonads in Relation to the Method of Preliminary Treatment Before Transplantation to the Recipient

Storage of grafts before transplantation	Total number of experiments	Number of experiments with indeterminate results		Number of experiments in which absorption of the graft took place	Number of survivals	
		result not known	result indefinite		Number of cases	%
No preliminary treatment carried out	20	1	–	15	4	21.1
Preserved in the cold without the use of isotonic solutions	19	–	1	12	6	31.6
Preserved in the cold in fluid from the eye	20	–	2	13	5	27.8
Preserved in the cold in blood serum	17	–	1	11	5	31.3
Preserved in the cold in amniotic fluid from embryos	18	1	1	9	7	43.8
Preserved in the cold in allantoic fluid from embryos	22	3	2	9	8	47.1

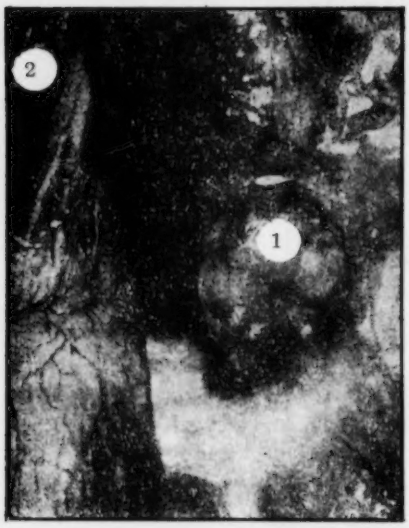

Fig. 1. The left oviduct of chick No. 089, displaced to the right side of the body. Its infundibulum (2) is found below the right lung, near a surviving grafted ovary (1) on the right lobe of the liver.

Fig. 2. Development of a transplanted testicle (a) in the same area as a regenerated ovary (b).

EXPERIMENTAL RESULTS

The results of our experiments may be judged from the figures given in Tables 1 and 2.

It can be seen from Tables 1 and 2 that in the experiments where no preservation of the grafts was carried out, their survival rate was comparatively low. On comparing the number of survivals in the different series of experiments it is evident that preservation of the grafts in allantoic and amniotic fluids of embryos had a favorable effect on their survival as compared with those preserved in fluids such as the fluid from the eye, which evidently possesses no property for promoting survival of grafts other than that of isotonicity. Even perservation of the grafts in blood serum, not only a natural isotonic solution but also an excellent nutrient medium for dividing cells, gave a lower survival rate than similar treatment of the grafts with amniotic and allantoic fluids from embryos. Healthy survival of grafts, as obtained in several experiments using the latter method, may be illustrated by Figs. 1 and 2.

SUMMARY

The transplants of the gonads of chicks aged from 18 days to 2.5 months were preserved in the allantoic and the amniotic fluids previous to transplantation. It was demonstrated that the percentage of take of these transplants is much greater than that of the transplants preserved in the aqueous humor, blood serum, or at low temperatures without any biological media.

LITERATURE CITED

[1] A. A. Vasil'ev and E. A. Sel'kov, Novyi Khirurg. Arkh. 33, 3, 333-345 (1935).

[2] G. E. Valdimirov, A. I. Govorov, A. I. Pelishenko and Z. A. Raiko, Collected Papers of Surgery, pp. 5--16, Leningrad (1948).*

[3] E. V. Dmitrenko, Biull. Eksptl. Biol. i Med. 37, 1, 59-61 (1954).

[4] L. N. Zamanskii, A. I. Lopushanskii and P. Ia. Siver, Doklady Akad. Nauk SSSR 99, 1, 177-181 (1954).

[5] L. N. Zamanskii and A. I. Lopushanskii, Ibid. 85, 3, 665-668 (1952).

[6] G. D. Knaizeva, Arkh. Patol. 15, 2, 41-50 (1953).

[7] G. P. Korotkova and L. S. Priezzheva, Vestnik Leningrad. Univ. Ser. Biol. 7, 3-21 (1952).

[8] G. P. Korotkova, Proceedings of a Meeting of Embryologists in Leningrad, pp. 171-173, Leningrad (1948).*

[9] M. S. Livshits, Collected Papers on Surgery, pp. 35-38, Leningrad (1948).*

[10] A. L. Rotenberg, Ibid. pp. 16-19.

[11] M. A. Sreseli, Ibid. pp. 21-28.

[12] V. P. Filatov, Tissue Therapy, Tashkent, (1948).

[13] M. Iu. Epshtein, Movyi Khirurg. Arkh. 6, 24, 449-526 (1925).

* In Russian.

CHARACTERISTICS OF THE SECRETION FROM DIFFERENT PORTIONS OF THE INTESTINE IN DOGS

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The attention of physiologists has long been directed towards the fact that the intestinal juice consists of two components: a liquid and a solid part, given the name of "mucous granules" [9]. The solid portion of the intestinal juice was formerly ascribed the role of acting as a framework for the feces. It was later discovered in the laboratory of the physiology of digestion of the Institute of Nutrition that the bulk of the enzymes: enterokinases, phosphatases, peptidase, lipases and saccharases – are secreted as components of the solid portion of the intestinal juice [10, 11, 8, 3], which was also confirmed by histochemical investigations [6].

N. P. Shepoval'nikov further found [9] that the solid portion of the secretion of the small intestine contains occasional degenerating cells from the intestinal epithelium, cholesteril crystals and pigments. L. S. Fomina [8] confirmed that the solid portion of the intestinal juice consists mainly of desquamated, disintegrating intestinal epithelial cells from the mucous membrane, and in addition contains a small number of lymphocytes and polymorphonuclear leucocytes. M. I. Razumov [4] described the morphological aspects of the processes concerned with the formation of the solid portion of the intestinal juice in dogs.

The secretion of enzymes by the large intestine has received very little study until recent times [1, 7]. We were unable to find in the literature any information about the secretion of the large intestine.

Only recently one of us [2] carried out an investigation of the juice of the caecum and the middle part of the large intestine, by the use of quantitative methods of enzyme estimation. The main results obtained in this experiment are given below.

In the present work our aim was to make comparative histological investigations of the secretion obtained from different divisions of the intestine, both small and large.

EXPERIMENTAL METHOD

The work was carried out on 9 dogs, with portions of various divisions of the intestine isolated by the Thiry method: in 3 animals segments of the duodenum and caecum were isolated; in 2 others, the duodenum and the middle part of the large intestine and in 4 animals, segments of the lower portion of the ileum.

The intestinal juice obtained during periodical secretion was examined. Its enzyme content: enterokinase, alkaline phosphatase, saccharase, lipase and peptidase – was determined by the methods adopted in the laboratory of the physiology of digestion [8, 12]. For the morphological examinations, intestinal juice was fixed without centrifuging in Zenker's fluid, formalin and alcohol. The fixed granules of the juice were embedded in paraffin wax. Sections, 6 μ in thickness, were stained with hematoxylin-eosin and thionine, and the distribution of desoxyribonucleic acid (DNA) was studied also, for which purpose Feulgen's histochemical reaction was used. Altogether 17 portions of juice were fixed and treated from the duodenum, 20 from the small intestine, 20 from the caecum and 12 from the middle portion of the large intestine.

EXPERIMENTAL RESULTS

During secretion without any local mechanical stimulation, in 5 hours from 0.2 to 1 ml of juice is produced by the caecum and by the isolated segment of the middle part of the large intestine – from 0.15 to 1.35 ml. The juice from both parts of the large intestine consists of a transparent fluid with greyish yellow granules and an admixture of a gelatinous, transparent mucus. The liquid and solid portions of the juice in these divisions are usually in the proportion of 1 : 1. Since the volume of juice during periodic secretion is very small, for the investigation of the individual enzyme contents of the liquid and solid portions we used juice obtained as a result of local mechanical stimulation.

TABLE 1

Enzyme Content of Intestinal Secretion (in units of 1 g) Obtained by Local Mechanical Stimulation

Dog No.	1		2		3		4	
Liquid or solid portion of juice	liquid	solid	liquid	solid	liquid	solid	liquid	solid
Amount of secretion in 5 hours (in g) . .	0,5	0,45	2,5	0,45	2,05	0,5	4,15	1,8
Phosphatase	300	2 400	185	1 500	350	3 930	300	3 370
Peptidases	0,0	120	0,0	41	14,5	82	7,2	50
Lipase	0,0	33	0,0	0,0	traces	52	traces	50

It can be seen from Table 1 that the bulk of the alkaline phosphatase, peptidase and lipase in the caecum and the middle part of the large intestine is secreted as components of the solid portion of the juice. This fact suggests that the process of enzyme secretion in the large intestine is of the same character as in the small intestine, consisting of the accumulation of enzymes in the epithelial cells. These then become desquamated into the lumen of the bowel, where they undergo disintegration and the enzymes are liberated into the chyme.

TABLE 2

Quantity of Enzymes in 1 g of the Solid Portion and the Quantity of Enzymes Produced During 1 Hour's Secretion From Various Areas of the Intestine During Periodic Secretion

Composition of secretion	During secretion for 1 hour				Enzyme content of 1 g of mucous granules			
	caecum		duodenum		caecum		duodenum	
	limits of variation	mean	limits of variation	mean	limits of variation	mean	limits of variation	mean
Phosphatase	96—380	187	1350—8100	5070	500—3620	2107	11000—54000	32300
Peptidase	5,1—9,1	7,0	39,8—398	114,9	42,0—95,9	69,8	239—1199	616
Lipase	1,6—5,0	3,0	22,2—132	64,0	25,0—105,6	56	180—600	378
Saccharase	0,04—0,26	0,15	0,23—1,94	0,75	—	—	—	—
Liquid portion	0,0—0,13	0,06	0,15—1,62	0,57	—	—	—	—
Mucus granules	0,04—0,13	0,09	0,06—0,32	0,18	—	—	—	—

However, the solid portion of the juice of the caecum contains 15-20 times less phosphatase and 10 times less lipase and peptidase than the solid portion of the duodenal juice. The rate of separation of the enzymes into

the composition of the juice of the caecum was also many times lower than that into the composition of the duodenal juice (Table 2).

The same relationships were observed also during a comparison of the duodenal juice with the juice secreted by the isolated segment of the middle part of the large intestine (Table 3).

TABLE 3

Quantity of Enzymes in 1 g of Mucous Granules and During 1 Hour's Secretion in Various Areas of the Intestine During Periodic Secretion

Composition of secretion	During secretion for 1 hour				Enzyme content of 1 g of mucous granules			
	middle part of the large intestine		duodenum		middle part of the large intestine		duodenum	
	limits of variation	mean	limits of variation	mean	limits of variation	mean	limits of variation	mean
Phosphatase . .	33,7—1 340	232	6 120—12 260	8670	940—8 930	2 650	16290—72000	39 870
Peptidase . . .	3,6—21,8	12	58—123,6	90,5	36,2—109	68,3	156—1060	452
Lipase	3,8—9,4	6,6	35,7—102,1	59,1	47—56,4	51,7	121—695	280
Saccharase . .	0,05—0,27	0,17	0,4—0,94	0,69	—	—	—	—
Liquid portion . . .	0,0—0,11	0,09	0,25—0,65	0,45	—	—	—	—
Mucus granules . . .	0,02—0,25	0,08	0,09—0,39	0,24	—	—	—	—

The morphological investigations showed that the solid portion of the intestinal juice from the duodenum and the lower part of the ileum have the same structure. It consists of accumulations of desquamated epithelial cells in various stages of disintegration, and of a small number of lymphoid cells. Sometimes in the mass of the

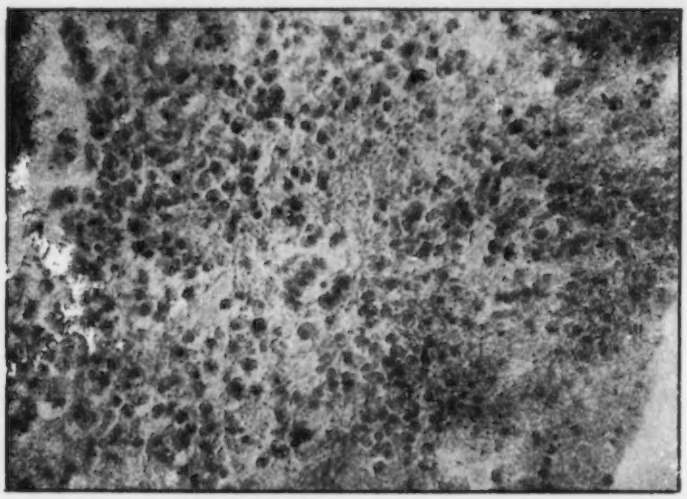

Fig. 1. Duodenal secretion in the experiment dated July 10, 1955. Stained with hematoxylin-eosin. Magnification 3 × 40. In the center of the film is seen a layer of epithelial cells which are not yet destroyed.

solid portion of the intestinal juice can be seen a small number of cells which have not yet been destroyed. They are arranged in the form of a layer or of a fragment of an epithelial tube, as described by M. I. Razumov (Fig. 1).

The nuclei of these cells often keep their normal size, intensity of staining and arrangement of chromatin, and the cells themselves retain their prismatic shape and well stained protoplasm. In the process of further conversion in the lumen of the intestin, the nuclei of the epithelial cells may retain their structure even when the protoplasm is destroyed. In contrast to the epithelial cells, the cytoplasm of the lymphoid cells as a rule does not stain, but the nucleus and the arrangement of the chromatin inside it are well preserved.

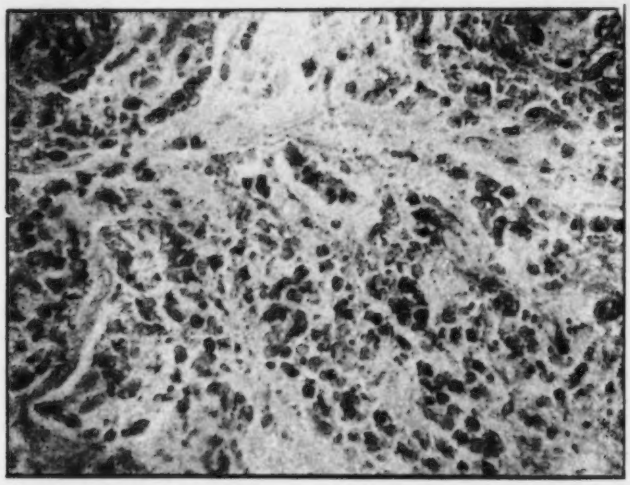

Fig. 2. Secretion of the caecum.
Stained with hematoxylin-eosin. Magnification 3 × 40. Cytoplasm well preserved. Cells arranged on a homogeneous fibrous background.

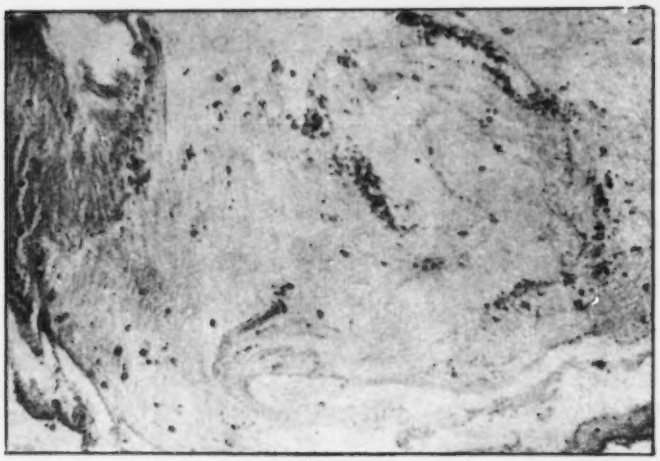

Fig. 3. Secretion of the middle part of the large intestine.
Stained with hematoxylin-eosin. Magnification 3 × 20. Occasional scattered cells are seen against a homogeneous background.

On the fixed preparations the greater part of the secretion has the appearance of an aggregation of granular protein masses, staining intensively with eosin and consisting of products of disintegration of the protoplasmic part of the cells. Amidst the granular protein framework nuclei are arranged, sometimes evenly, sometimes densely. The majority of nuclei are reduced in size, often shrunken, and staining intensively and continuously with basic dyes. In addition, in the mass formed by disintegration of the protoplasm of the cells granules are distributed, sometimes having the appearance of droplets. All these formations are products of rupture of the cell nuclei. They stain intensively with hematoxylin and give a clearly positive reaction for desoxyribonucleic acid (DNA). On treatment of the films by Feulgen's method, in some portions of the secretion the individual stages of destruction of the nuclei can be traced. In place of the nucleus several granules of nuclear material appear in the cell, distributed in the cytoplasmic part of the cell. The number of these granules may vary. In far advanced processes of autofermentation of the cells, the nuclei are shattered into tiny granules having the appearance of droplets. The nuclear membrane ceases to be distinguishable and the droplets lie quite evenly throughout the cell. When the margins of the cells are destroyed, they are distributed throughout the whole mass of the juice, including the liquid portion, where they can be found by staining with hematoxylin and by the Feulgen reaction for DNA. M. I. Razumov [5] reported similar findings. Sometimes the nuclear granules and droplets merge with each other, forming clumps of nuclear material in the form of large spheres. These clumps give a positive reaction for DNA. Their formation may possibly be associated with processes of autofermentation caused by delay in secretion of the solid portion of the juice.

The investigations show that the solid portion of the juice from the caecum, like the solid portion of the juice from the samll intestine, is an accumulation of desquamated, disintegrating cells from the epithelial lining, together with a few lymphoid cells. In this juice many of the nuclei also are apparently disintegrating into separate fragments of various sizes or into fine droplet-like granules. Individual stages in the splitting up of the nuclei may also be observed. Sometimes the DNA of the nuclei of the solid portion of the juice in this division of the intestine also forms small clumps in the form of spheres 2-3 times larger than the nucleus, or rod-like formations. However, in the solid portion of the caecal juice secreted periodically, it is never possible to see nuclei with a normal distribution of chromatin and with the characteristic intesnsity of staining. The majority of the nuclei are pyknotic and are shaped like angular clumps.

The cytoplasm of the cells in the juice from the caecum is better preserved than in the juice from the small intestine. It always stains intensively and has sharply outlined contours. Sometimes the contours of the protoplasm of the cells are well outlined even when their nuclei are completely absent. The cells themselves in the cecal secretion lie separately, and sometimes are unevenly distributed among the homogeneous fibrous mass that forms a background (Fig. 2). After treatment of this secretion with thionine a comparatively large amount of mucus is revealed in the form of quite large bands and threads. This is hardly observed at all in the juice of the small intestine.

The juice from the middle part of the large intestine is distinguished morphologically from the caecal juice by the fact that in the solid portion of the juice of this division of the intestine, areas are often observed in which few cells are found. The cells lie at great distances from each other, distributed among a large volume of mucus which has a homogeneous fibrous structure or appears in the form of thick bands and threads (Fig. 3).

In many samples of juice obtained from isolated segments of various divisions of the intestine, small collections of polymorphonuclear leucocytes are observed. These cells may possibly find their way into the juice from the outer margin of the orifice of the fistula.

The structure of the liquid portion of the intestinal juice is the same in the histological preparations from all divisions of the intestine. It has the appearance of a collection of protein material in the form of fine granules, among which may sometimes be seen isolated cells and droplets of nuclear substance.

SUMMARY

Ferments – phosphatase, lipase and peptidase are contained in the compact part of the large intestinal secretion. The compact portion of secretion from the isolated section of duodenum, ileum, caecum and the medial portion of the large intestine consists of detached epithelial cells (at various stages of disintegration), mucus and lymphoid elements. However, the compact part of secretion is characterized by its own morphological peculiarities in each portion of intestine. Much more cellular elements are contained in the compact part of duodenal and small intestinal secretion than in the compact portion of secretion of the caecum and the medical portion of the large intestine. The latter contains much mucus and the cytoplasm is better preserved. However, there are no nuclei with the normal structure revealed.

LITERATURE CITED

[1]. G. B. Berlatskii, The Physiology of the Large Intestine, Dissertation, St. Petersburg (1903).*

[2] I. B. Kuvaeva, Proceedings of the Tenth Scientific Session of the Institute of Nutrition of the AMN SSSR, pp. 44-45, Moscow, (1956).*

[3] S. Ia. Mikhlin, Proceedings of the Session of the Institute of Nutrition of the AMN SSSR on February 3-7, 1949, pp. 18-20, Moscow (1949).*

[4] M. I. Razumov, Voprosy Pitaniia 11, 4, 18-28 (1952).

[5] Idem, Ibid. 12, 3, 33-45 (1953).

[6] B. K. Skirko, Ibid. 6, 38-47 (1953).

[7] N. D. Strazhesko, The Physiology of the Intestines Dissertation, St. Petersburg (1904).*

[8] L. S. Fomina, Problems of Nutrition, 1, pp. 139-151, Moscow (1951).*

[9] N. P. Shepoval'nikov, The Physiology of the Intestinal Juice, Dissertation, St. Petersburg (1898).*

[10] G. K. Shlygin, Papers Presented at the Seventh All-Union Congress of Physiologists, Biochemists and Pharmacologists, pp. 406-408, Moscow (1947).*

[11] Idem, Uspekhi Sovremennoi Biol. 33, 1, 14-32 (1952).

[12] Idem, Biokhimiia, 15, 6, 509.

* In Russian.

MORPHOLOGY AND PATHOMORPHOLOGY

INTERNEURANAL CONNECTIONS OF THE EXTERNAL GENICULATE BODY OF THE CAT

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The question of interneuronal connections in the central nervous system has not been adequately studied in spite of a large number of investigations. This also applied perfectly to a number of problems relating to the conducting pathways in the central nervous system and to the study of the synaptic endings themselves. The study of the interconnection between the different divisions of the visual analysor has for a long time attracted the attention of research workers. There are many reports in the literature dealing with the structure of the external geniculate body and also with its connections with the peripheral part of the visual analysor – the retina – and with the visual area of the cortex.

The most complete investigations on the structure of the external geniculate body are those by Thuma [18], Rioch [16] and others. These workers showed that the external geniculate body consists of two nuclei – dorsal and ventral. In the process of phylogenesis the importance of the dorsal nucleus increases and in it terminates the bulk of the optic fibers. Depending on the species of animal, the dorsal nucleus consists of a varying number of cell strata. In respect of the ventral nucleus there is still no unanimity in the literature. On the question of whether optic fibers terminate in the ventral nucleus, the findings are contradictory. The connections of the external geniculate body with the retina have been studied both by the method of cell degeneration [3, 12, 13 and others] and by the method of degeneration of fibers. Minkowski [12, 13] showed that the direct and crossed optic fibers terminate in different strata of the dorsal nucleus. His observations, based on the method of transneuronal degeneration of cells, were subsequently confirmed by the findings of L. Ia. Pines and I. E. Prigonnikov [3]. On the basis of findings obtained by the use of Marchi's method of degeneration of fibers, the retina is projected in the external geniculate body as an inverted image. The direct and crossed fibers terminate in different parts of the dorsal nucleus.

With the problem of the place of termination of the direct and crossed fibers in the external geniculate body is directly connected that of the mode of termination of the optic fibers in this particular formation. Ramón-y-Cajal [15], using Golgi's method, found that the optic fibers terminate in the geniculate body of the cat in free racemose ramifications around the cells of the dorsal nucleus. Other workers [7, 8], using in their experiments on cats, rabbits and monkeys special methods for demonstrating synapses, showed that the fibers of the optic tract do not terminate in free endings; they form synaptic endings on the bodies and dendrites of cells. These authors studied the interneuronal connections of the external geniculate body of the cat in normal and experimental conditions and demonstrated the presence of synapses but made no attempt to relate these to the cell architecture of this formation. However, a more complete idea of the interneuronal connections may be obtained on the basis of comparison of the distribution of synapses in different portions of the external geniculate body with the cell architecture of this particular formation.

The object of the present investigation was to study the interneuronal connections of the external geniculate body of the cat in normal and experimental conditions and to compare the results obtained with the cell architecture of the external geniculate body.

EXPERIMENTAL METHOD

The material used in the investigation was the external geniculate bodies from normal cats and from cats after operation. In the experimental animals the optic nerves, chiasma and optic tracts were also investigated. The operation consisted of section of the optic nerve. The animals were killed on the 2nd, 3rd, 4th, 6th and 7th days after operation. The material was treated by the following methods: 1) Nissl's, 2) Ramón-y-Cajal's, after preliminary fixation for 24 hours in chloral hydrate, 3) the Golgi-Deinek method and 4) the Bielschowsky-Gros method. Sections were cut to a thickness of 20-25 μ.

In studying the experimental material the Golgi-Deinek and Bielschowsky-Gros methods were used.

EXPERIMENTAL RESULTS

Structure of the external geniculate body. On the basis of the study of a series of sections the cell structure of the external geniculate body of the cat may be represented as follows. The main mass of cells is the dorsal nucleus, which is present in all the sections, starting from the oral pole and ending at the caudal. The ventral nucleus appears only on certain sections near to the caudal pole and occupies only a small area in relation to the dorsal nucleus. During a study of a series of sections from the oral to the caudal pole, the dorsal nucleus is seen to be in three layers. According to the classification of Minkowski [13], these three layers are called the external peripheral layers, the internal peripheral layers and the central layers. In all these layers the presence of 3 types of cell – large, medium and small – may be observed. In the ventral nucleus are mainly to be found transitional forms from medium to small cells. Within the bounds of the dorsal nucleus, as the series is studied in a caudal direction, projection fibers may be observed in the dorsolateral aspect of approximately the middle third of the external geniculate body. Between these fibers are scattered large ganglion cells. In these same sections, dorsal to the bundle of projection fibers appears a small group of cells, which form an area of the dorsal nucleus separated from the main cell mass by the projection fibers. It must be pointed out that in the cat, at approximately the level of the oral division there is a reticular layer which surrounds the external geniculate body. In this layer are scattered nerve cells of medium size.

In the preparations of the external geniculate body of the normal cat, stained by the methods of Ramón-y--Cajal, Golgi-Deinek and Gros -Bielschowsky, it can be seen how the optic fibers enter the external geniculate body. The presence of terminal endings of characteristic appearance is seen on the body and dendrites of the cells of all the layers of the dorsal nucleus. As a rule these are loops or small rings, round or oval in shape. Sometimes synapses are met in the form of a special type of reticulum. In the majority of cases a connection is seen between the terminal ring or loop and the preterminal ramification of the fiber. In Fig. 1, a is illustrated a cell of the dorsal nucleus of the external geniculate body of the cat. As can be seen, the fine preterminal branches approach the cell and terminate on its body and dendrites in rings, loops or peculiar ring-like endings. In some cases synapses are also observed outside the connections with the preterminal fibers. In Fig. 1, b is shown a cell of the external geniculate body, situated between projection fibers.

Characteristically on these cells a comparatively large number of synaptic endings is almost always found. In Fig. 1, c is illustrated a cell with synaptic endings as revealed by the Bielschowsky-Gros method.

The number of synapses in our sections for one cell of the external geniculate body varies from 2 to 40; these variations are probably due to the fact that impregnation of the synapses depends on the their functional stage at the time of fixation. Synapses are distributed over the whole surface of the cell and its dendrites; they are particularly numerous at the point of emergency of the processes from the cell body. The diameter of the synapses on the cells of the external geniculate body varies from 2.5 to 3μ.

Degenerative changes in the external geniculate body of the cat after section of one optic nerve. Two days after section of one optic nerve, individual cells situated in the central layer of the dorsal nucleus of the ipsilateral external geniculate body show a slight increase in size,as compared with normal,of their argentophillic synapses (Fig. 2, a). However, in addition to these there are also some quite normal endings. In the opposite external geniculate body no altered synapses are to be found after 2 days. In studying the sections at this stage we were unable to find any destructive changes whatsoever in the optical fibers themselves.

On the 3rd day (see Fig. 2, b) after section of the optic nerve, degenerative changes are observed in the synapses on cells situated in the central layer of the ipsilateral external geniculate body, and also in the peripheral

Fig. 1. Normal synaptic endings on the cells of the external geniculate body. a) On a cell of the dorsal nucleus. Ramón-y-Cajal's method. Objective 90, ocular 15; b) on a cell between the projection fibers. Golgi-Deinek method. Objective 90, ocular 15; c) on a cell of the dorsal nucleus. Bielschowsky-Gros method. Objective 90, ocular 15.

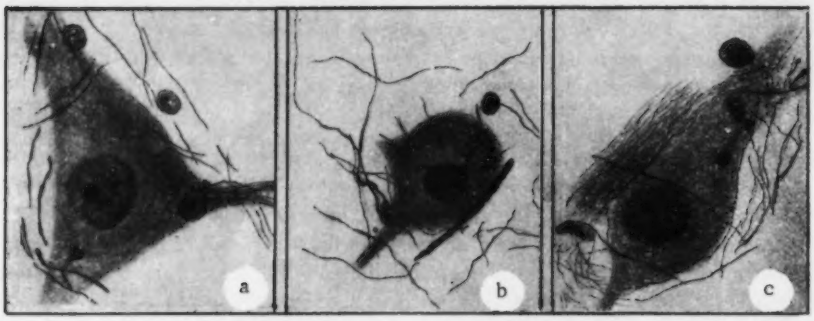

Fig. 2. Various stages of degeneration of synaptic endings on the cells of the dorsal nucleus.
a) Two days after section of the optic nerve. Bielschowsky-Gros method. Objective 90, ocular 15; b) 3 days after section of the optic nerve. Bielschowsky-Gros method. Objective 90, ocular 15; c) 4 days after section of the optic nerve. Bielschowsky-Gros method. Objective 90, ocular 15.

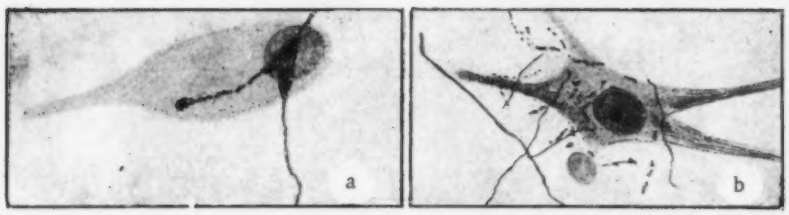

Fig. 3. Degenerating synaptic endings and fibers in the dorsal nucleus.
a) Four days after section of the optic nerve. Bielschowsky-Gros method. Objective 90, ocular 15; b) 6 days after section of the optic nerve. Bielschowsky-Gros method. Objective 90, ocular 15.

layers of the contralateral external geniculate body. Degeneration of the synapses is shown by a progressive enlargement of the synapses as compared with normal, and by their greater argentophilia. In some cases the cavity of the synapse is almost completely impreganated with silver. At this stage of degeneration the optic fibers have already undergone considerable alteration; their silver-staining properties are increased; they are slightly thickened as compared with normal, and a small proportion of the fibers in the ipsilateral external geniculate body have undergone partial fragmentation. In some sections it may be seen how one optic fiber divides into 3 or 4 branches. Synapses are also found lying in the intercellular space.

On the 4th and 6th days after section of the optic nerve terminal endings can be seen in all stages of degeneration. This fibrils are still present, terminating in normal synapses; however the majority of the terminal loops and rings are now converted into solidly stained bulbs with uneven contours, and within the individual endings the presence of fine granules is seen. In Fig. 2, c is illustrated a cell from the central layer of the dorsal nucleus of the ipsilateral external geniculate body, on which are seen both degenerating ending with a granular substance within, and normal rings. The size of the degenerating synapses greatly exceeds that of the normal synapses. At this stage completely fragmented synapses are observed on individual cells of the peripheral layer of the dorsal nucleus of the contralateral external geniculate body, and moreover the fragmentation extends as far as the preterminal fiber (Fig. 3, a). Furthermore the majority of the fibers entering the external geniculate body are undergoing severe fragmentation (see Fig. 3, b). Synapses are observed in different stages of degeneration, mainly on the medium-sized cells. A large number of degenerating synapses is also found on the cells of the reticular layer. In addition to these, normal synaptic endings may be observed on the large cells of both ipsi- and contralateral external geniculate bodies.

The entire picture of degeneration of synapses and fibers applies to cells and fibers situated in the central and peripheral layers of the external geniculate body on the operated and also on the opposite side. It should be mentioned that during the first 3 days after operation, degeneration was seen most sharply in the ipsilateral external geniculate body. In the ventral nucleus, neither in normal nor in experimental conditions was it possible to find any synaptic endings. In the same way we were unable to detect degeneration of synapses on the cells situated between the projection fibers.

The question of the interneuronal connections in the external geniculate body of the cat is mainly connected with the character of the endings of the optic fibers. Our findings in respect of the endings of the optic fibers in the external geniculate body of the normal cat, based on methods of demonstration of synapses, suggest that the opitc fibers form endings on the bodies and dendrites of the cells of the dorsal nucleus of the external geniculate body, shaped like loops, rings or reticula, splitting up into separate fibers. In addition synaptic endings are found on the cells lying between the projection fibers and on the cells of the reticular layer of the external geniculate body.

During examination of the shape of the normal synapses it was most usual to observe loops or rings of a round, oval or elongated shape. Sometimes synapses in the form of reticula were seen. Identical shapes of endings are also seen in other divisions of the central nervous system in mammals [1, 2, 5, 14, etc.] and man [11, etc.].

It was shown that a cell of the external geniculate body of the cat may have a large number of contacts with the optic fibers; in some cases up to 40 synapses may be observed on the body of the cell and its dendrites. In our sections it was not possible to estimate directly whether this number corresponds to one optic fiber or whether each fiber terminates in only one synapse, since on account of the thinness of the sections the preterminal branch could not be traced for a long way from the synapse. However, in some cases the initial division of the optic fiber may be seen at its point of departure from the tract; this suggests that one optic fiber has endings on several cells.

By comparing the findings on interneuronal connections with the cell architecture of the dorsal nucleus we found the presence of synapses in all the layers on large, medium and small cells. Synapses were discovered, too, on cells situated between the projection fibers and on the cells of the reticular layer.

In consequence of section of one optic nerve, we observed at different intervals of time after the operation degenerative changes in the synapses, mainly on middle-sized cells situated in the dorsal nucleus of the external geniculate body on both the side of operation and the oposite side. A similar pattern of degeneration was observed by Glees and Clark [8] and Glees [7] in the external geniculate body of the monkey and cat, and also by M. P. Frolova [4], Hoff [10], Gibson [9], Schimert [17], Auer [6] and others in various divisions of the central nervous system.

Comparison of the areas of degeneration with the cell architecture of the external geniculate body showed that after section of one optic nerve, degenerative changes are observed in synapses and fibers situated mainly in the central part of the ipselateral external geniculate body and in the peripheral parts of the contralateral external geniculate body. The presence of solitary normal synapses on the 6th day after operation, on the large cells of both external geniculate bodies and the absence of degenerative changes in the synapses on the cells between the projection fibers leads us to postulate that these endings belong to some other afferent system. These may also be the endings of afferent fibers leading, according to several workers, from the occipital lobe of the cortex to the external geniculate body, and finally, they may belong to local connections. This question cannot be considered to be finally settled.

From the investigations described the following conclusions may be drawn:

1. Synaptic endings are normally found on all 3 types of cell in the dorsal nucleus of the external geniculate body and also on cells situated between the projection fibers.

2. The number of synapses in contact with a cell body and its dendrites may reach 40.

3. After section of the optic nerve, at different intervals of time after operation degenerative changes are observed in synapses situated mainly on the medium-sized cells of the dorsal nucleus of both the ipsi- and contralateral external geniculate bodies. Degenerating synapses are found in large numbers on the cells of the reticular layer.

4. On the basis of the study of degeneration of synapses and fibers in the external geniculate body after section of the optic nerve, it may be supposed that the optic fibers not only terminate in the different parts of the dorsal nucleus, but are also to some extent connected with the reticular layer.

SUMMARY

The cytoarchitectonics and the interneuronic connections of the external geniculate body was studied in normal conditions as well as in section of the optic nerve. The synapses were revealed on the 3 types of cells of the dorsal nuclei and on the cells located between the projection fibers. After section of the optic nerve the degenerative changes were noted in the synapses and the fibers of various portions of the dorsal nucleus in the external geniculate body. These changes were revealed on both sides. Degenerating synapses were also found on the cells of reticular layer. It is assumed that the optic fibers terminate not only on the cells of the dorsal nucleus but are to a certain extent connected with the reticular layer.

LITERATURE CITED

[1] B. A. Dolgo-Saburov, Zhur. Vysshei Nerv. Deiatel., 4, 6, 903-908 (1954).

[2] A. D. Zurabashvili, Synapses and Reversible Changes in Nerve Cells, Moscow (1951).*

[3] L. Ia. Pines and I. E. Prigonnikov, Problems of the Morphology of the Cerebral Cortex, pp. 57-99, Moscow (1936).*

[4] M. F. Frolova, The Importance of Interneuronal Connections in Maintaining the Structure of the Neurone, Dissertation, Moscow (1955).*

[5] T. I. Entin, Arkh. Anat. Gistol. i. Embriol. 31, 4, 25-32 (1954).

[6] J. Auer, J. Anat. 1956, V. 90, p. 30-40.

[7] P. Glees, J. Anat. 1941, V. 75, p. 239-247.

[8] P. Glees and W. E. L. Clark, Ibid. p. 295-307.

[9] W. G. Gibson, Arch. Neurol. a. Psychiatr. 1937, V. 38, p. 1145-1157.

[10] W. S. Hoff, Proc. Ray. Soc. Biol. Sc. 1932, V. 111, p. 226-237.

[11] J. Minkler, Arch. Neurol. a. Psychiatr. 1941, V. 45, pp. 44-45.

[12] M. Minkowski, Arb. a. d. Hirnanat. Inst. in Zürich, 1911, Bd. 7, S. 225-362.

* In Russian.

[13] Idem, Schweiz. Arch. Neurol. u. Psychiatr. 1920, Bd. 6, S. 201-252.

[14] G. S. Phalen and H. A. Davenport, J. Comp. Neurol. 1937, v. 68, p. 67-81.

[15] S. Ramón-y-Cajal, Histologie du systeme nerveux de l'homme et des vertebres, Paris 1911.

[16] D. Rioch, Ibid. 1929, v. 49, p. 1-119.

[17] J. Schimert, Ztschr. Anat. 1938, Bd. 108, S. 761-767.

[18] B. D. Thuma, J. Comp. Neurol. 1928, v. 46, p. 173-198.

THE INFLUENCE OF THE NERVOUS SYSTEM ON THE MICROSTRUCTURE OF THE GLANDULAR TISSUE OF THE MAMMARY GLAND

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The experiments of M. M. Mironov [2] on the denervation of the udder in goats led I. P. Pavlov [3] to the view that the nervous system influences the activity of the mammary gland. He also postulated a trophic influence on the activity of the mammary gland, although up to the present no histological investigations have been carried out in support of this hypothesis.

Research is particularly lacking on the importance of the nervous system for the structure and formation of the parenchyma of the mammary gland. Elucidation of this aspect of neurogenic influence may be an important factor in understanding the complex process of the physiology of lactation.

EXPERIMENTAL METHOD

We studied the influence of the nervous system on the structure of the mammary gland by the method of denervation of the gland and subsequent histological investigation. The spermatic nerve and its branches were divided; the vessels were treated with 5% cabolic acid to inactivate the vascular nerves. In order to secure more complete isolation of the mammary gland from its main nerve connections, the skin incision around the gland was made in two stages and the whole gland freed from adjacent tissues. Great attention was paid to keeping the blood supply of the gland intact.

In the present paper we describe the results of experiments carried out on the mammary glands of guinea pigs undergoing operation in a state of sexual immaturity – aged from 1 to $1^1/_2$ months. For histological investigation the mammary gland of the guinea pig has a great advantage over those of large animals. Guinea pigs have a development cycle of short duration, they multiply rapidly and easily, and they possess well developed glandular tissue which corresponds in its structure to the mammary glands of higher animals. Of the 37 sexually immature guinea pigs undergoing operation we took the mammary glands from 18 for morphological investigation when the animals were in a typical sexually immature state; 9 guinea pigs were in various stages of pregnancy at the moment of fixation of the glands, and 10 were in the period of lactation. The mammary glands of the guinea pigs undergoing operation were taken for histological examination at various stages of development. For fixation we used Zenker's fluid and 12% formalin; in some cases celloidin, in others paraffin wax was used for embedding; sections were stained with Mayer's hemalum or Böhmer's hematoxylin with counterstaining with eosin, and iron hematosylin with couterstaining with Mallory's mixture. In addition, control investigations of the denervation were carried out by the Bielschowsky-Lavrent'ev silver method.

EXPERIMENTAL RESULTS

In a guinea pig $1^1/_2$ months old the mammary gland tissue is in its initial stage of development (Fig. 1, A). It has the appearance of a small number of large, slightly branched ducts, terminating in ill-defined end portions.

The epithelium of the ducts and the end portions consists of narrow, high cells with oval and elongated nuclei. The borders of the cells are ill-defined. The lumen of some of the ducts contains a secretion which on the fixed preparations has a friable appearance.

The epithelial tubes are surrounded by a small, discrete layer of fibrous connective tissue which gives way to septa subdividing the mass of fatty tissue which forms the main basis of the gland. The structure of the denervated gland in the same animal 15 days after operation does not differ from that of the control gland (Fig. 1, B). The slightly branched epithelial tubes terminate in ill-defined end portions. The epithelium is of the same type throughout. Connective tissue is present mainly around the epithelial tubes and in the form of fine septa subdividing the fatty tissue.

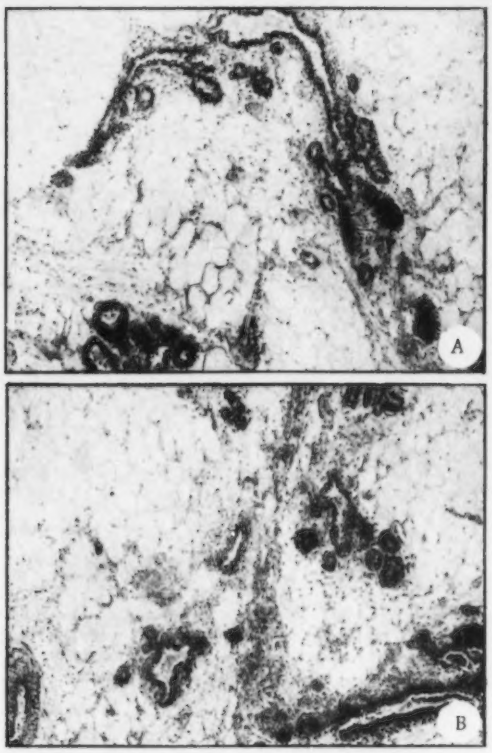

Fig. 1. Normal (A) and denervated (B) mammary gland of guinea pigs at the age of $1^1/_2$ months. Stained with hematoxylin-eosin. Objective 15, ocular 5 ×.

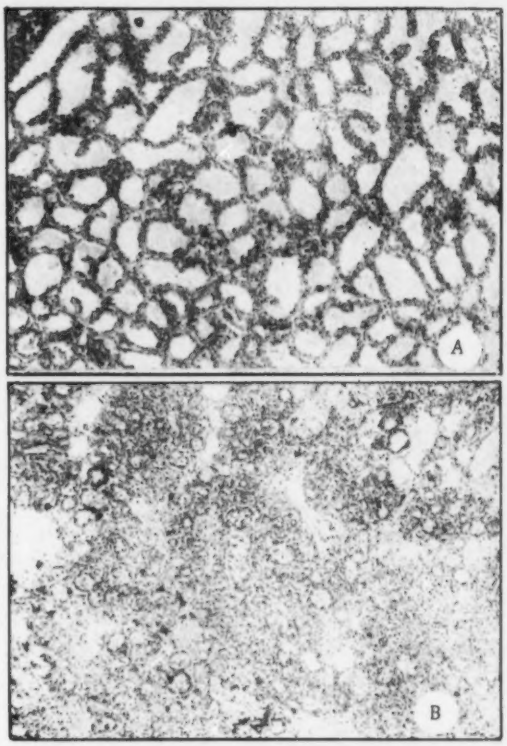

Fig. 2. Normal (A) and denervated (B) mammary gland of a guinea pig on the second day of lactation. Stained with iron-hematoxylin and Mallory's mixture. Objective 15, ocular 5 ×.

The structure of the glandular tissue showed quite a different picture after denervation carried out on sexually immature animals, if their mammary glands were taken for histological investigation at various periods of pregnancy or lactation. At the onset and in the middle of pregnancy the changes observed mainly took the form of retardation of development of the glandular tissue of the denervated mammary gland.

At the end of pregnancy more pronounced, changes were observed in the structure of the parenchyma of the gland characterized by delay in formation of the mammary glands deprived of their main nerve connections. The tissue of control glands of these animals consists of clearly formed glandular lobules, separated by narrow connective tissue septa. Each lobule contains a large number of alveoli, a considerable proportion of which have cavities filled with secretion. Alveoli or various shapes – from spherical to elongated – are lined with cubical epithelium with round or slightly oval nuclei. Side by side with alveoli of this type are seen alveoli without cavities.

They are formed of low epithelial cells, in close contact with each other, and containing eleongated nuclei. The efferent ducts are moderatly distended by the secretion with which they are filled. In places the epithelium of the ducts is in a single layer and is very much thickened. The interlobular connective tissue is clearly shown and is formed here and there of more compact or looser fibrous tissue.

The glandular tissue of the denervated glands in this group of animals is less well developed than in the control group. This is shown in the first place by the predominance of connective over glandular tissue. Alongside the relatively formed alveoli there are branching epithelial tubes without clearly defined alveoli. The majority of the alveoli are without lumen, showing their inactive condition. The alveolar epithelial cells are most often cubical and contain oval or round nuclei, occupying the greater part of the cell. In only a few alveoli is the lumen filled with secretion. The epithelium of these alveoli is cylindrical or cubical, with round nuclei. The lumen of the efferent ducts are very dilated; in cross section they are irregular, sometimes stellate in shape. The double layer of epithelim of the ducts is condensed and the cell nuclei are situated parallel to the lumen. The latter is filled with secretion and cells.

Particularly severe changes in the structure of the glandular tissue following denervation of the mammary glands of the sexually immature guinea pigs are observed during lactation of the animals. These changes are associated with abnormal formation of glandular tissue and are expressed by disturbance of the normal development of the gland and of the course of the secretory process. An example of this group of experiments is the mammary gland of a guinea pig undergoing operation at the age of 1 month and killed $14^{1}/_{2}$ weeks later, on the 2nd day of lactation.

Normally the mammary gland of a lactating guinea pig shows well-developed glandular tissue (Fig. 2, A). The majority of the alveoli are very dilated and filled with milk. The epithelial cells are large and, depending on the state of secretion, vary in shape from cubical to flattened. The epithelial cells contain round nuclei; sometimes cells containing two nuclei are found. Alongside functioning lobules are seen lobules in a resting state. The lumen of the efferent ducts are filled with milk; their epithelium is flattened and their nuclei elongated. The interlobular and interalveolar connective tissue is in the form of fine, tense septa. All this is characteristic of the gland in a state of active secretion.

The denervated gland of this guinea pig differs appreciably from the control (Fig. 2, B). It contains a large number of nonfunctioning lobules. The alveoli in the lobules are greatly altered and have a very polymorphic appearance. In the majority of cases the epithelium of the vesicles shows some degree of degenerative change. Alveoli with epithelium showing relatively little change are rarely encountered. In some alveoli there is an appearance of desolation: the vesicles are in a resting state and the lumen is not distinguishable. In other cases the lumen of the vesicles are preserved but their epithelium is low, with flattened nuclei, situated directly by the lumen; this indicates the absence of a regenerative process after apocrine secretion. In some alveoli the epithelium shows a return to a more simplified pattern. Now and then it can be seen that fat droplets are formed in certain cells but are not excreted, so that these cells have a distended appearance and their nucleus is pressed towards the basal side. The alveoli with a lumen are often filled with contents, not ordinary milk but an altered secretion, with a coarse granular appearance in the films, sometimes with remains of desquamated epithelial cells. The ducts are stretched, and lined with flattened epithelium. Between the collagen fibers there is a considerable amount of amorphous material, giving the loose connective tissue of the interlobular septa a somewhat edematous appearance.

From the results obtained we are able to confirm that removal of the main nerve connections from the mammary glands of sexually immature animals is reflected mainly in the subsequent development of the glands at the most active periods of their formation and activity. Besides general failure to develop or delay in the formation of the parenchyma of the denervated gland in relation to the normal functional state of the animal, disorders are observed in the course of the secretory process. These disorders are mainfested both in degenerative processes affecting the epithelium of the alveoli and ducts and in interference with the expulsion of droplets of fat from the secreting cells. As a result an accumulation of fat in the epithelial cells takes place; they take on a swollen appearance with rounded apical edges, which is a sign of absence of the normal outflow of secretion into the lumen of the alveoli.

Investigation of the denervated glandular tissue in the preactive period of its formation, i.e. in the sexually immature guinea pig, showed no abnormal tendencies. Thus, denervation of the mammary gland has no immediate

effect on the structure of the glandular tissue of the sexually immature animal. However, at periods when the activity of the mammary gland normally appears, the denervated gland is seen to develop imperfectly, on account of the unpreparedness of the glandular tissue for normal functioning. This unpreparedness is the consequence of disturbance of certain intimate processes taking place in the mammary gland in the postnatal period, requiring the participation of the nervous system for their effection. Here is clearly seen the trophic influence of the nervous system in the development of the glandular tissue, without which the normal formation of the mammary gland is impossible.

Our findings are in agreement with the results of the research of A. G. Ginetsinskii et al. on denervation of the kidney [1] and of L. A. Chudnovskii on denervation of the ovary [4]. These authors point out that denervation of the organ in the early postnatal period causes it to develop imperfectly, with disturbance of its subsequent activity.

SUMMARY

The author describes the results of experiments on the denervation of the mammary gland in guinea pigs with subsequent histological study. Thirty-seven sexually immature guinea pigs were operated. The mammary glands were taken for examination in 18 sexually immature guinea pigs, in 9 – at various periods of pregnancy and in 10 – during lactation. The denervation in sexually immature guinea pigs does not cause any direct changes in the structure of the mammary gland up to the period of preparation to lactation. Both, during the latter period and during lactation the operation of denervation of the mammary gland alters considerably its histological structure. The above experiments show the pronounced effect rendered by the nervous system on the normal function of the mammary gland.

LITERATURE CITED

[1] A. G. Ginetsinskii, I. I. Likhnitskaia and E. L. Semenova, Biull. Eksptl. Biol. i Med. 34, 2 (1952).

[2] M. M. Mironov, Arkh. Biol. Nauk 3, 4 (1895).

[3] I. P. Pavlov, Cited from: Complete Collected Works, Vol. 2, Izd. AN SSSR, (1940).*

[4] L. A. Chudnovskii, Trud. inst. Fiziol. AN SSSR 4, 1955.

* In Russian.

NEUROVASCULAR RELATIONSHIPS IN THE WALL OF THE STOMACH DURING INTERFERENCE WITH THE PORTAL BLOOD FLOW

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The present paper is devoted to a description of the neurovascular relationships in the wall of the stomach during interference with the blood flow in the portal venous system in man (in cirrhosis of the liver) and during ligation of the portal vein in animals. It is the sequel to our earlier research in this direction [16].

EXPERIMENTAL METHOD

In order to study the neurovascular relationships in the wall of the stomach material was utilized from the cadavers* of 5 persons dying from cirrhosis of the liver and from 20 animals (cats and dogs) after ligation of the portal vein. The animals survived for one to eight months after the experiment. We used the fundus and the pyloric division of the stomach for the investigation. The specimens were treated mainly by the Bielschowsky-Gros method, with counterstaining of the vessels with acid fuchsin; Ranson's and Nissl's methods were also used.

The nervous apparatus of the stomach, especially in animals [12], is impregnated with great difficulty. Despite the use of various methods of fixing and staining the material, we did not succeed in obtaining a clear picture of the nerve plexuses of the cat's stomach by means of the Bielschowsky-Gros and Ranson methods. We were able to see only nerve trunks with regenerating fibers and cells of a round shape and devoid of processes.

The nervous apparatus of the dog's stomach impregnates poorly but more completely, we obtained the best results from staining the human stomach.

EXPERIMENTAL RESULTS

During examination of histological preparations of the above-mentioned portions of the stomach from man and animals, the first to be observed is the severe dilatation of the capillaries, the density of the vascular network and the presence of blood cells in the surrounding tissues.

The neurovascular relationships which we found are as follows. In the first place, very often and mainly in preparations from the human stomach, dendrites of nerve cells – particularly of Auerbach's plexus – with bulbs (or spherical thickenings) at their ends are situated on adjacent vessels (Fig. 1). Sometimes the majority of dendrites of a cell are included in a connection of this sort. In the second place it is often possible to see synaptic endings of fine fibrils situated on the walls of vessels (Fig. 2). These endings are of the most varied shapes and sizes. The presynaptic fibers have either quite even contours or, more often, they are uneven and contain pools of neuroplasm along their course. Finally it may also be possible to seen the picture of a nerve fiber, at the point of its intersection of a capillary, giving off lateral processes shaped like rockets of irregular disks (Fig. 3). The latter form of neurovascular relationships was mainly observed in dogs.

* The postmortem material was placed at our disposal by D. S. Sarkisov (of S. S. Vail's laboratory) and the Department of Pathologic Anatomy of the S. M. Kirov Order of Lenin Military Medical Academy.

Fig. 1. Intermuscular nerve plexus in the human stomach. Connections of the dendrites of nerve cells with capillaries.
1) Capillary; 2) dendrite with "pin-head" at the end. Stained by the Bielschowsky-Gros method. Magnification: ocular 15×, objective 90×.

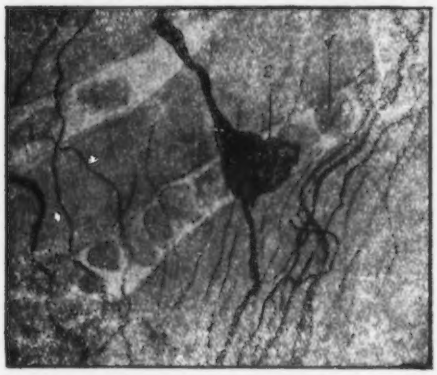

Fig. 3. Intermuscular nerve plexus of the stomach of the dog. A lateral process of the nerve fiber at its point of intersection with a capillary.
1) Capillary; 2) lateral process of the nerve fiber. Stained by the Bielschowsky-Gros method. Magnification: ocular 15 ×, objective 90 ×.

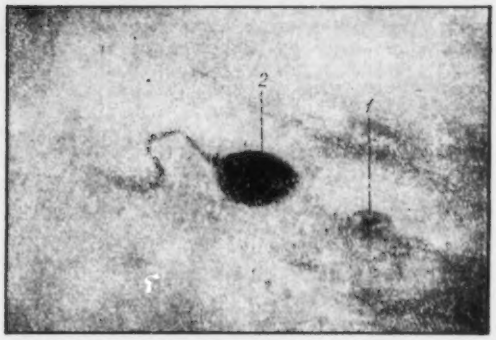

Fig. 2. Intermuscular nerve plexus in the stomach in man. A synaptic ending on a vessel.
1) Vessel; 2) synaptic ending. Stained by the Bielschowsky-Gros method. Magnification: ocular 10 ×, objective 90 ×.

Connections between dendrites and vessels were previously described in the central nervous system by Golgi [17], who postulated that it was through these connections that the nutrition of the parenchyma of the brain is effected. This view was supported by a number of workers, but later on M. D. Lavdovskii [13] and others categorically denied that connections could exist between dendrites and capillaries, believing that they could occur only in the glial cells.

Dendrites are usually regarded as a means for the enlargement of the receptive surface of the neuron and for increasing its area of contact with the axon of the adjacent neuron which forms pericellular structures at this point. In recent years some neurohistologists have begun to regard the role of the dendrites as being wider [12].

Z. N. Khoros [15], investigating neurones in ganglia in the vermiform appendix and ileocaecal region in man, the rabbit and the cat, found that one cell may give off processes whose endings lie on the most varied structures, including vessels. On these grounds this worker considers that the dendrites of the nerve cell have different physiological characteristics.

On the subject of the physiological role of dendrites terminating on vessel walls, it may be supposed that neurovascular connections of this type are of importance in the performance and regulation of metabolic processes, especially in pathologic conditions such as cirrhosis of the liver when considerable biochemical changes take place in the composition of the blood.

These problems may be finally solved by means of histochemical methods of investigation. So far as the second and third types of neurovascular connections are concerned, the first question which arises here is the nature of these nerve endings on the vessels, i.e. it has to be determined from what fibers they are formed – preganglionic or postganglionic.

From his extensive observations on axonal synapses on vessels in the central nervous system, B. A. Dolgo-Saburov [6] considers that they are conductive in origin, i.e. of the same nature as the synapses observed on the dendrites and bodies of nerve cells. A. S. Gusev's preliminary experimental findings confirm this hypothesis.

The synapses which we found on the vessels of the stomach are evidently formed from preganglionic fibers of the vagus nerve. This assumption is confirmed by the presence of synapses on the Dogiel type one cells in the wall of the stomach and duodenum and their absence from the middle portion of the small intestine. Here we found only connections between dendrites and vessels [16] and not once did we see synapses on capillaries like those described above. It is natural to think that the synapses found on the Dogiel type one cells and on the vessels of the stomach have a common origin. The detailed analysis of this question will be the subject of our research in the future.

The synapses on the vessels of the stomach are evidently present in normal conditions too, but pathological processes cause them to undergo reactive changes, leading to argyrophilia, and thereby making them more readily demonstrable.

D. Iu. Guseinov and S. Rustambekov [3] investigated the nervous apparatus of the stomach and described ramifications of nonmedullated fibers on the capillaries, forming retiform sheaths with large number of thickenings and "boutons terminaux." In the wall of an arteriole also they found a nerve end-plate (in the opinion of these authors it was probably receptor) closely connected with its cells.

N. N. Zhuravlev [7], who studied the nervous appartus of the stomach in man in carcinoma, saw that where they intersect with the capillaries, nerve fibers form varicose thickenings much larger than those usually seen along the nerves.

If the illustrations in certain papers on the pathomorphology of the vegetative nervous system [2, 8] are examined, nerve endings may be observed on vessels; the authors did not, however, give this feature its due recognition.

There is no unanimity in the literature on whether the nervous system in the different divisions of the gastrointestinal tract, for example the stomach and the small intestine, is equally vulnerable. S. S. Vail' [1], who investigated the nervous apparatus of the stomach and intestines in tuberculosis, came to the conclusion that in comparison with the ganglia of the small and large intestines, the nervous apparatus of the stomach is particularly vulnerable. On the other hand, N. G. Kolosov [11] is of the opinion that the nervous apparatus of the intestine is most delicate and vulnerable.

In our investigations of the ganglia of the wall of the stomach and ileum in man and experimental animals we found the most varied and pronounced changes in the nerve cells in the ileum. Such features were found less often when we studied the nervous apparatus of the stomach wall; when they were observed there it was only in the submucosal plexus and Auerbach's plexus in the pyloric region. We usually found changes in the nerve structures in places where the cells were mainly of Dogiel's second type; in our opinion they are less resistant to any form of change in the internal environment of the body.

Our findings are in contrast to those of certain workers [9, 10 and other] who consider that cells of Dogiel's first type undergo the greatest changes during pathological conditions. The same opinion is held by N. G. Kolosov [11], who explains the great vulnerability of Dogiel's type one cells by their greater differentiation.

If we agree with N. G. Kolosov, however, it is difficult to account for the fact that in the same pathological condition (cirrhosis of the liver) almost half the ganglion cells in the intermuscular plexus of the small intestine (not to metnion the submucosal plexus) are severely affected, whereas in the stomach these changes are only slight.

SUMMARY

The author examined the intestinal wall in 5 cadavers of patients deceased from cirrhosis and in 20 animals (cats and dogs) after the ligature of their portal veins. Tissues were treated by Bielschowsky-Gros method with an additional staining of the blood vessels by acid fuchsin. Intimate neurovascular relations were revealed in the liver both in human cirrhosis and in experimental animals (dogs) after the ligature of the portal vein. These relations were represented by the presence of terminations of dendritis of the ganglionic cells on capillaries. Large numbers of synaptic endings of thin fibers (of various length) were also found on the blood vessels.

LITERATURE CITED

[1] S. S. Vail', Arkh. Patol. Anat. 3, 1, 32-37 (1937).

[2] D. N. Vyropaev, Problemy Tuberk. No. 4, 50-60 (1945).

[3] D. Iu. Guseinov and S. Rustambekov, Azerbaidzhansk. Med. Zhur. No. 9, 69-73 (1956).

[4] B. A. Dolgo-Saburov, Zhur. Vysshei Nerv. Deiatel. 4, 6, 903-908 (1954).

[5] Idem, Doklady Akad. Nauk SSSR, 103, 3, 521-524 (1955).

[6] Idem, Proceedings of the 1st Belorussian Conference of Anatomists, Histologists, Embryologists and Topographical Anatomists, pp. 84-85, Minsk (1957).*

[7] N. N. Zhuravlev, Ibid., pp. 95-96.

[8] A. N. Kartavenko, Transactions of the Smolensk Medical Institute, Vol. 3, pp. 247-250, (1950).*

[9] M. F. Kirik, Morphology of the Autonomic Nervous System, pp. 179-199, Moscow (1939).*

[10] I. I. Kolesnichenko, Collection in Memory of A. V. Leontovich, pp. 324-329, Kiev (1948).*

[11] N. G. Kolosov, Innervation of the Internal Organs and Cardiovascular System, Moscow-Leningrad (1954).*

[12] Idem, Vestnik Leningrad. Univ. Ser. Biol. No. 21, 42-55 (1956).

[13] M. D. Lavdovskii, Russkii Vrach. Mo. 17, 649-653 (1902).

[14] T. N. Radostina, Some Problems of the Innervation and Vascularization of the Intestine, Author's Abstract of Disseration, Moscow (1954). *

[15] Z. N. Khoros, Proceedings of the 1st Belorussian Conference of Anatomists, Histologists, Embryologists and Topographical Anatomists, pp. 388-389, Minsk (1957).*

[16] M. V. Shepelev, Ibid. pp. 355-356.

[17] C. Golgi, Sulla fine anatomia degli organi centrali del sistema nervosa, Melano (1886).

* In Russain.

METHODS

THE METHOD OF BALLISTOCARDIOGRAPHY IN CHRONIC EXPERIMENTS ON DOGS

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As a method of clinicophysiological investigation of the contractile function of the myocardium, ballistocardiography has been widely used abroad, and in recent years it has begun to be developed in the Soviet Union [1-3, 5, 6].

The use of this method experimentally on animals is necessary also when observations are being carried out on the hemodynamic manifestations of experimental lesions of the cardiovascular system (experimental arteriosclerosis, myocardial infarction, artificial defects of the heart and so on), and in the solution of a number of cardinal problems of the theory of ballistocardiography a whole host of difficulties is encountered. These are mainly concerned with the necessity of ensuring complete muscular relaxation of the animal at the time of recording the ballistocardiogram (BCG). In consequence of this, the majority of the existing methods of experimental recording of the BCG are used in acute experiments on anesthetized laboratory animals [4].

It is clearly of the greatest interest to clinical physiology to be able to record the BCG during a chronic experiment. In such cases repeated recordings on the same animals would be possible, and consequently observations of a dynamic character would be facilitated on animals in various physiological states or at different stages of development of an experimental pathological condition. From this point of view Darby's method [7], with which the BCG can be recorded in chronic experiments on dogs, is in our opinion the most promising.

Darby's method is as follows. Into the spinous processes of T_6-L_5 of an anesthetized dog are inserted nylon threads by means of which a curved aluminum rod, 25-40 cm long and weighing 40-60 g, is attached to the spine; the rod projects 15 cm beyond the sacrum and to its end is attached an Arbeit feeder. The dog is placed prone, its head is supported and its spine is made as flat as possible. The dog lies on a rubber sheet.

According to the authors' paper, the BCG recordings made 2 weeks after insertion of the nylon threads were inferior in unanesthetized dogs to anesthetized, since many artefacts were observed because of muscular contractions and respiration. Thus, among the disadvantages of this method are such complicating factors as insertion of nylon threads operatively into the spinuous processes of the vertebral column under anesthesia, and the occurrence of numerous artefacts during recording of the BCG on unanesthetized dogs.

While engaged in developing a method of recording certain hemodynamic indices in chronic experiments on dogs, we discovered a means of obtaining a BCG of perfectly satisfactory quality (Fig. 1) in unanesthetized dogs. For recording the BCG we used a Dock electromagnetic feeder. The feeder is fixed to the animal's body as follows: a duralumin plate, bent to the shape of the animal's spine, is firmly fixed to the vertebral column and sacrum by rubber bands (Fig. 2); there are tags along the edge of the plate to which the rubber bands are attached; these pass beneath the abdomen of the animal to the opposite side of the plate; at its wide part the plate is fixed to the sacrum by a rubber band passed under the groin and fixed to the plate on the same side again.

At the end of the wide portion of the plate are mounted the coils of the Dock feeder, made of organic glass. These are fixed by side pieces of the same material to an organic glass plate which is attached by copper screws to the duralumin plate. In order to minimize the risk of breakage of the feeder by accidental movement of the

Fig. 1. Ballistocardiogram of an unanesthetized dog. (Recording made with a 4 PFD 7 apparatus for functional diagnosis).
Explanation of tracings (from above down): ballistocardiogram of rate, electrocardiogram (lead II), respiration, time marker (1 second).

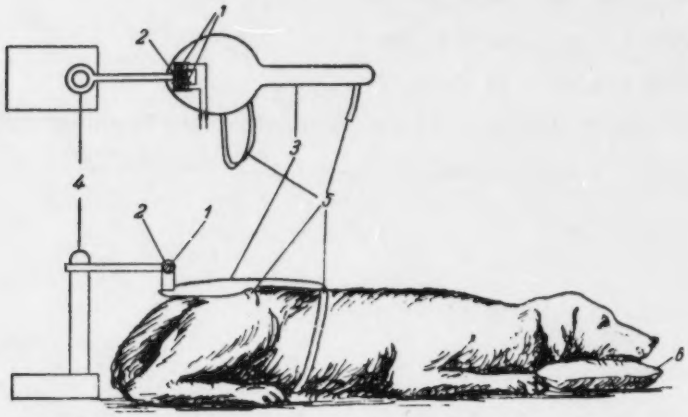

Fig. 2. Position of the animal and feeder during recording of the BCG. 1) Feeder coil; 2) flat magnet; 3) aluminum plate fixed to the region of the sacrom; 4) stand for the magnet; 5) rubber bands fixing the plate to the body of the animal; 6) sandbag pillow.

dog, the distance between the coils is increased to 17 mm. Further, to maintain adequate sensitivity of the feeder, the number of turns in the coil windings must be taken to 12,000. The feeder projects 3 cm beyond the border of the sacrum. The dimensions of the duralumin plate are as follows: over all length of the plate 36 cm, width of the narrow portion 6 cm and of the wide portion 20 cm, thickness 1.1 mm. Weight of the plate and feeder 235 g.

The experiment is preceded by a definite preparation of the animal. Dogs designated for BCG recording are accustomed to lying prone on a sand tray with their spine extended. For this purpose a sandbag is placed under the animal's head so that its forepaws are situated at the side of the sandbag (See Fig. 2).

By the method described above, a bent duralumin plate of the given dimensions, without feeder, is fixed to the spine and sacrum of the animal. On the sand behind the dog, on a firm stand, is mounted a flat magnet. After

2-3 weeks of such training, when placed on the familiar apparatus, the animal lies in what has become a usual pose, perfectly motionless and with its muscles relaxed, which permits the BCG to be recorded.

It is desirable for the work to be carried out in a room which is insulated from outside stimuli. This shortens the period of preparation and improves the quality of the BCG recording. We performed the experiments in a conditioned reflex chamber, and whereas the dog was placed in the chamber, the experimenter and the recording equipment were in an adjoining room.

The method suggested for direct ballistocardiography during chronic experiments on dogs is simple and does not require the animals to be anesthetized before the recording of the BCG is made. The need for a special operative procedure is eliminated.

Drawbacks of the method include the necessity for preliminary preparation of the animal and also the fact that during the experiment, the operator must have an assistant near the animal.

SUMMARY

The method which is suggested in this paper permits to record the ballistocardiogram of nonanesthetized dogs in chronic experiments using the Dock's electromagnetic feeder. If the dogs are trained beforehand to lie in the necessary position with a feeder fixated on the sacrum it is not necessary to anesthetize them.

LITERATURE CITED

[1] R. M. Baevskii, Voen.-Med. Zhur. No. 5, 29-32 (1956).

[2] A. I. Gefter, A. P. Matusova and S. S. Belousov, Terap. Arkh. 29, 7, 26-32 (1957).

[3] Lin Ch'eng, Klin. Med. No. 9, 124-131 (1957).

[4] A. V. Mareev, Biull. Eksptl. Biol. i Med. No. 1, 105-108 (1958).

[5] V. V. Parin, Klin. Med. 34, No. 6, 12-24 (1956).

[6] V. V. Parin and A. V. Mareev, Atherosclerosis and Coronary Insufficiency, pp. 235-248, Moscow (1956).*

[7] T. D. Darby, L. I. Goldberg and P. C. Gazes, et al., Proc. Soc. Exper. Biol. a Med. 1954, V. 86, pp. 673-676.

* In Russian.

THE DB-1-57 STEREOTAXIC APPARATUS OF THE INSTITUTE OF NORMAL AND PATHOLOGICAL PHYSIOLOGY OF THE AMN SSSR

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In recent years the stereotaxic technique first suggested by Horsley and Clarke in 1908 [2] has been used on an ever-increasing scale in electrophysiological and morphological investigations of the central nervous system, particularly of the subcortical formations.

Abroad a number of models of stereotaxic apparatus are available, mainly adapted for research on cats, rabbits and small monkeys.

Among these may be mentioned the Gaze and Gordon apparatus [1]; apparatus developed in the USA (see J. Neurophysiol. for the last few years, beginning with 1950); the French apparatus with Dell's modification;* the Loewenfeld and Altman apparatus, and others.

Besides the stereotaxic apparatus working with rectangular coordinates by the Horsley-Clarke system, special instruments are available also which allow the electrode to be directed at a predetermined angle (in a tangential system). One such model was suggested by Harrison [3] in 1938.

The main requirements of any stereotaxic apparatus are as follows:

1. Fixation of the animal's head to correspond with the zero plane of the Horsley-Clarke coordinate system.

2. A manipulator to provide movement of the electrode in three mutually perpendicular planes with an accuracy of not less than one tenth of a millimeter.

However in addition to these fundamental requirements each model possesses a number of devices which increase the scope of the apparatus and facilitate its use. Among these are, for instance, the presence of two or sometimes four manipulators for the introduction of two or four electrodes respectively; a firm and massive stand to fix not only the head but the animal as a whole, which is most essential to avoid vibration, undesirable when working with buried electrodes, and others.

We succeeded in planning and constructing a suitable stereotaxic apparatus to meet modern requirements. The prototype DB-1-57 apparatus was made in the experimental workshops of the institute and tested in the laboratory of general physiology.**

Brief Description of the Apparatus

The stereotaxic apparatus DB-1-57 of the Institute of Normal and Pathological Physiology of the AMN SSSR is constructed in the form of a massive stand for fixation of the whole animal (usually cats). The base of the apparatus consists of a carrier frame, which is composed of two longitudinal steel bars (guides), connected by two triangular steel crosspieces (Fig. 1).

*Manufactured by La Precision Cinematographique, France.

**We have now developed a second model of the stereotaxic apparatus (DB-2-58), a description of which will be published later.

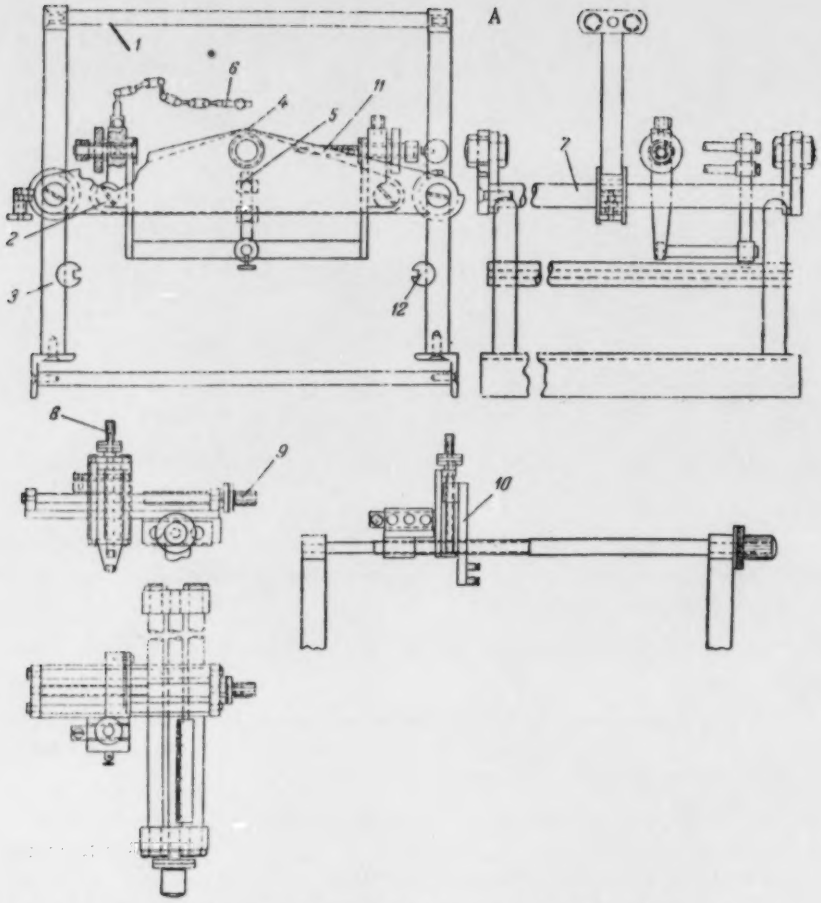

Fig. 1. General outline of the stereotaxic apparatus and manipulator.
1) Guide for the manipulator as a whole; 2) turning frame; 3) main upright; 4) bearing for rotation of the central frame; 5) support for the head-piece; 6) hinged electrode holder; 7) main carrying frame; 80 limb of the "x" axis; 9) limb of the "y" axis; 10) electrode carrier; 11) ear holder; 12) runners for the heated table.

The carrier frame is firmly connected to the base of the apparatus by means of four steel uprights, the base consisting in its turn of two longitudinal steel angle girders and four round steel tie bars. Parallel to the longitudinal bars of the carrier frame and slightly below it are a further two firmly attached bars, which increase the rigidity of the construction and at the same time act as guides for the heated table on which the animal lies (Fig. 1).

The heated table slides freely in the grooves of these bars and can be withdrawn backwards in case of necessity. The base of the apparatus is covered with duralumic sheet, on which is placed a trough with a wastepipe.

The longitudinal bars of the carrying frame act as guides for the manipulator which is attached to two clamped sliding bushes. Consequently, the manipulator may be moved along the whole length of the animal's body, from the beginning of the head to the end of the trunk. Therefore in this model the manipulator may be used also for investigations in the whole of the spinal cord which alone greatly widens the scope of the apparatus.

For the convenience of the experimentor (during preparation and so on), the manipulator can be retracted on the left side through an angle of 120° (Fig. 3).

The manipulator itself is made in the form of a ⊓-shaped bridge, whose vertical sides are massive T-shaped steel bars with reversible steel shackles, by which the manipulator is attached to the sliding bosses of the carrying frame (see Fig. 1).

To the T-shaped bars are fixed two transverse, round steel guides, on which slides a brass runner, permitting movement of the electrode in a sagittal plane. Displacement of the runner is carried out by turning a transverse screw, with a pitch of 1 mm, which is also fixed to the T-shaped bars. The graduated scale of this screw is divided into 100 parts, so that when the scale is deflected through 1 division, the runner (or electrode) is displaced through 10 μ in the sagittal plane. The range of the runner to right and left of the zero line is 50 mm. To this "sagittal" runner is attached a steel rectangular plate with sidepieces, also with round steel guides and a screw to allow displacement of a further brass runner in the frontal plane for a distance of 40 mm forwards and backwards from the zero position, with an accuracy of 10 μ.

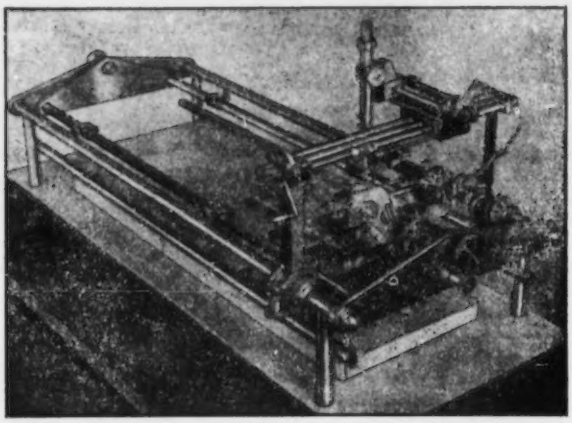

Fig. 2. The DB-1-57 stereotaxic apparatus in its working position.

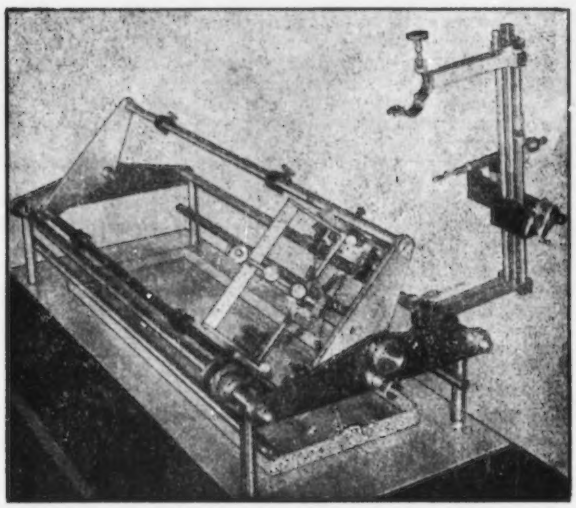

Fig. 3. The same: manipulator retracted, inner frame rotated, head support strongly inclined.

To the "frontal" runner in turn is fixed a vertical arm, to which is attached a texolite plate. To this plate is fixed a brass boss with a screw, equipped with a special micrometer to allow movement of the electrode in a vertical direction. The micrometer is freely movable in this boss upwards and downwards for 60 mm, and may be securely fixed at any level by the screw. In this way the coarse movement of the electrode is achieved. By turning the graduated scale of the micrometer a fine movement of the micrometer rod may be obtained for a distance

of 60 mm in an upward and downward direction with an accuracy of 10 μ. At the end of the micrometer rod there is a threaded hole with a screw, to which is attached the hinged electrode-holder or a special platform with a cathode repeater for working with a microelectrode.

Thus, the manipulator can produce movement of the electrode in three mutually perpendicular planes with an accuracy of 10 μ.

In our model, the animal is fixed in a special frame which is placed inside the main carrier frame of the apparatus. To this inner frame are attached the head support and 4 arms for fixation of the limbs (the limbs are tied with straps to the 4 arms of this frame). This inner revolving frame, in contrast to the outer (carrier) is not firmly secured and can turn freely through 360° on two semiaxes, together with the affixed animal (see Fig. 3).

The construction of the inner revolving frame is identical with that of the outer.

Into the triangular crosspieces of the outer frame are inset brass bearings which receive the semiaxes emerging from the triangular crosspieces of the inner revolving frame. Into the ends of these semiaxes, from the outer side, are inserted strong screws which, when tightened, fix the inner frame in any desired position. In addition, in the side of the outer triangular crosspiece corresponding to the head end of the frame there is a futher counter-pin screw which serves for accurate fixation of the inner frame in the horizontal plane (parallel to the horizontal plane of the manipulator) in the working position, i.e. in the prone position of the animal (Fig. 2).

The possibility of turning the affixed animal during the course of the experiment provides free access to any part of the animal's body, which is extremely important during research connected with the study of cortico-visceral problems (see Fig. 3).

Another essential detail of the apparatus is the head support, which is constructed as follows: to the longitudinal bars of the inner frame are firmly fixed two vertical steel bars, the arms of the head support. Into the arms are screwed brass bosses which project slightly inwards. These bosses serve a dual function: 1) they are guides for the clamps of the external auditory meatus; these clamps are usually two round steel pegs with ebonite tips which are attached by means of a screw clip (see Figs. 1, 2 and 3); 2) they serve as bearings for rotation of the $\sqcap$-shaped frame of the head support around an axis passing through the external auditory meati.

On the crossbeam of the $\sqcap$-shaped frame there is a tailpiece along which moves a vertical bar which carries the clamp for the upper jaw. This clamp is made in the form of two pieces, each of which can move along the vertical bar.

The lower platform serves as a support for the upper jaw, and the upper, inclined platform presses on the dorsum of the nose. For better tightening of the nasal clamp from above a compression nut is screwed home.

This construction provides secure fixation of the animal's head.

The $\sqcap$-shaped frame of the head support may be fixed in any position on its bearings by two screws.

On the left side of the head support there is an inclinometer by means of which the animal's head can be placed at any angle to the horizontal plane (see Fig. 2).

Thus, without disturbind the principal of fixation of the head, the construction of the head support enables the head to be turned about the axis of the external auditory meati. This makes it possible to insert the electrode not only vertically but also at any angle (usually not more than ± 45°), i.e. to employ the tangential method. In addition the ability to incline the head downwards strongly makes working in the cerebellar region, and expecially the medulla, much easier.

These two features considerably widen the scope of this particular model.

Finally, the last constructional feature of the apparatus is an adaptor which permits simultaneous working with 8 buried electrodes (this adaptor can be used with success also for work with superficial electrodes).

In the upper part of the arms of the head support there is a hole in which are inserted round horizontal rods (along the axis of the apparatus), fixed with screw clamps. On these rods are set the flexible electrodes of the support, 4, on each side (8 in all). Each holder is constructed independently and the experimenter may arrange them in any combination (an equal number on each side, all on the same side, and so on).

The flexible electrode holders consist of two parts: a) the holder arm, fixed to the rod, with a texolite platform and a receptacle for the next part, and b) the holder itself, which is a flexible, hinged combination, terminating in a miniature spring clip. On this clip there is a shackle which, in the forward position clamps it and in the backward position releases it (see Figs. 1, 2 and 3).

By means of this clip the electrode is easily and firmly fixed in any position.

The flexible electrode holder is used as follows. At first the electrode, fixed in the grip of the manipulator, is inserted stereotaxically in the brain. Next, without releasing the grip of the manipulator, the tip of the flexible electrode holder is passed beneath it, and the projecting end of the electrode is seized and clipped. Afterwards the grip of the manipulator is released and the latter is withdrawn upwards. The electrode remains in situ.

By repeating this procedure up to 8 electrodes can be inserted in the brain by means of one coordinate manipulator.

It should be emphasized that during rotation of the animal, the flexible electrode holders rotate also and the position of the inserted electrodes is not disturbed (Fig. 3).

Thus, the ability to work with 8 buried electrodes at the same time is an essential feature of this particular model of stereotaxic apparatus.

SUMMARY

The authors present a description of a stereotaxic apparatus for cats which has the following advantages over the existing models:

1. A massive stand for fixation of the animal.
2. A device for rotating the fixated animal.
3. The possibility to bend the head of the animal in any direction.
4. The presence of elastic electrode-holders which permit to work simulatneously with 8 immersed electrodes.

LITERATURE CITED

[1] R. M. Gaze, and W. Gordon, Quart. J. Exper. Physiol. 1954, V. 39, 4, p. 279-304.

[2] V. Horsley and R. Clarke, Brain 1908, V. 31, p. 45.

[3] F. Harrison, Arch. Neurol. a. Psychiatr. 1938, V. 40, p. 563-565.

[4] I. Loewenfeld and R. Altman, J. Neuropath. a. Exper. Neurol. 1956, V. 15, p. 181-189.

THE ARGENTO- AND MERCURIMETRIC DETERMINATION OF THE CONTENT OF SH- GROUPS IN THE SERUM AND BRAIN HOMOGENATE OF THE CAT

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The amperometric method of titration of SH- groups with silver nitrate, introduced on 1946 by Kolthoff and Harris [7], has also been successfully applied to the analysis of several thiol compounds (cystein, glutathione and other) [8, 11], and to the determination of the content of SH- groups in different proteins [6, 10], in the blood serum [2, 12] and also in nonprotein blood filtrates and extracts of organs and tissues [3, 5]. K. V. Savich and V. A. Iakovlev [1] showed that it was possible to use this method in determining the content of SH- groups directly in a tissue homogenate. However Benesch, Lardy and Benesch [4] have recently expressed the opinion that titration of SH- groups of proteins with $AgNO_3$ in an ammoniacal buffer may give too low results because of "disappearance" of part (by oxidation) of the SH- groups by the action of the ammonium ion in an alkaline medium. Results confirming this point of view were obtained by the authors cited [4] and also by Ingram [6] during titration of the SH- groups of hemoglobin in a buffered solution containing 0.25 M NH_4OH and 0.05 M NH_4NO_3. In order to find out whether significant oxidation of the SH- groups of blood serum and brain homogenate does take place during titration with $AgNO_3$ in a mixed ammoniacal buffer, we carried out simultaneous titration of SH- groups with $HgCl_2$ in a phosphate buffer. Under these circumstances our findings differed from those of Kolthoff, Stricks and Morren [9], according to whom one mole of $HgCl_2$, during titration in a phosphate buffer in the presence of potassium chloride, combines with one mole of SH- groups in cystein, glutathione and serum albumin.

EXPERIMENTAL METHOD

Weighed samples of brain tissue (50-100 mg) were homogenized in a Potter glass homogenizer with 0.5 ml of distilled water, kept cold on ice; distilled water was then added to the homogenate until 1 ml contained 25--30 mg of tissue. Next, 1 ml of homogenate or 0.5 ml of serum was transferred to a titration vessel in which was placed 25 ml of buffered solution. When titrating the SH- groups with $1 \cdot 10^{-3}$ M $AgNO_3$ solution we used a buffered solution containing 0.01 M NH_4OH and 0.08 M NH_4NO_3. When titrating the SH- groups with $1 \cdot 10^{-3}$ M $HgCl_2$ solution, we used a 0.1 M phosphate buffer (pH = 7.34), containing 0.05 M KCl. The method of titration was fundamentally identical with that described in the paper by Kolthoff and Harris [7]. The current in the circuit was recorded by means of a type M-91 microammeter with a sensitivity of 10^{-7} amp/ deg. The rate of revolution of the platinum electrode was 750-800 rpm. Titration was carried out in aerobic conditions. The titer of the $AgNO_3$ solution was checked by Mohr's method, and the titer of the $HgCl_2$ solution by a gravimetric method based on precipitation of mercury with H_2S. All the solutions were made up in double distilled water, redistilled from a glass vessel.

EXPERIMENTAL RESULTS

In the Table are shown the results of the experiments (each figure therein presents the average of 2-3 parallel determinations).

We see that the results of argento- and mercurimetric determination of the content of SH- groups in the

serum and brain homogenate are in practically complete agreement. It can be concluded from these results that during titration of SH- groups with silver nitrate in a mixed ammoniacal buffer, no significant oxidation or "disappearance" of these groups evidently takes place by the action of ammonium ions, since titration with $HgCl_2$ in a phosphate buffer gives the same results. The agreement of the results of argento- and mercurimetric determination of SH- groups also indicates that one mole of SH- groups in the serum and brain homogenate during titration in a phosphate buffer containing KCl. This conclusion is in full agreement with the results of the above-mentioned investigations by Kolthoff, Stricks and Morren [9].

Comparison of the Results of Argento- and Mercurimetric Titration of SH- Groups in the Serum and Homogenate of the Cerebral Cortex of the Cat

Experiment No.	Content of SH-groups in μM	
	in 100 ml of serum	
	$AgNO_3$	$HgCl_2$
1	61,5	63,0
2	47,5	45,0
	in 100 mg of brain tissue	
3	1,20	1,10
4	0,95	0,93

In conclusion it should be mentioned that the amperometric titration of SH- groups in the serum and especially in a tissue homogenate with silver nitrate has, in our opinion, a number of advantages over titration with mercuric chloride. In the first place, in titration of the SH- groups with $AgNO_3$ the value of the current is more rapidly established in relation to a given quantity of added reagent. In the second place, titration with $AgNO_3$ can be done in the presence of ethylenediaminotetraacetic acid (EDTA), which has the property of stabilizing the SH- groups of thiol compounds. From our findings, the content of SH- groups in tissue homogenate to which EDTA was added in a concentration of $3 \cdot 10^{-5}$ M does not vary over 14 hours when stored at a temperature of about 0°C. In the same homogenate not containing EDTA, 11-12% of the SH- groups is oxidized during storage for 24 hours under the same conditions.

SUMMARY

A simultaneous titration of SH-groups by $HgCl_2$ in phosphate buffer was undertaken for the determination of the question whether a noticable oxidation ("disappearance") of SH-groups takes place in the blood serum and in the brain homogenate under the effect of ammonium ion in their titration by $AgNO_3$ in diluted ammoniac buffer. Experiments demonstrated that the results of the argento- and mercurimetric determination of the concentration of SH- groups in the blood serum and brain homogenate coincide completely. These data permit to conclude that in titrating the SH-groups by $AgNO_3$ in diluted ammoniac buffer there is no appreciable oxidation or their "disappearance" under the effect of ammonium ion.

LITERATURE CITED

[1] K. V. Savich and V. A. Iakovlev, Voprosy Med. Khim. 3, 2, 121-128 (1957).

[2] R. Benesch and R. E. Benesch, Arch. Biochem. 1948, Vol. 19, pp. 35-45.

[3] R. Benesch and R. E. Benesch, Ibid. 1950, Vol. 28, pp. 43.

[4] R. E. Benesch, H. A. Lardy and R. Benesch, J. Biol. Chem. 1955, Vol. 216,pp. 663-676.

[5] J. W. Goldzieher, W. B. Rawls and M. A. Goldzieher, Ibid. 1953, Vol. 203, pp. 519-528.

[6] V. M. Ingram, Biochem. J. 1955, Vol. 59, pp. 653-661.

[7] I. M. Kolthoff and W. E. Harris, Industr. a. Eng. Chem. Anal. Ed. 1946, Vol. 18, pp. 161-165.

[8] I. M. Kolthoff and W. Stricks, J. Am. Chem. Soc. 1950, Vol. 72, pp. 1952.

[9] I. M. Kolthoff, W. Stricks and L. Morren, Anal. Chem. 1954, Vol. 26, pp. 366-372.

[10] W. Staib and F. Turba, Biochem. Ztschr. 1956, Bd. 327, S. 473-483.

[11] W. Stricks , I. M. Kolthoff and N. Tanaka, Analyt. Chem. 1954, Vol. 26, pp. 299-305.

[12] N. Weissman, E. B. Schoenbach and E. B. Armistead, J. Biol. Chem. 1950, Vol. 187, pp. 153-165.

A NEW SELECTIVE METHOD OF STAINING MAST CELLS

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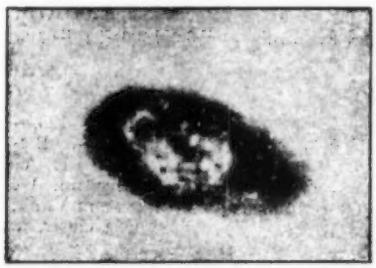
(Received February 20, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Because of the physiological importance of the mast cell and the consequent necessity of counting these cells in microscopic preparations and with the need to carry out further study of mast cells, some improvement in the histological methods used to detect them is desirable.



Mast cell in the subcutaneous connective tissue of a white mouse.
Stained by the method described above.
Magnification: objective 100 × immersion, ocular 10 ×.

We have devised a method of selective staining of the mast cell granules in sections and films. Any form of fixing is permissible which preserves mast cells (absolute alcohol, alcohol-formalin, Homgren's method with a 4% solution of basic lead acetate, Sylven's method with a mixture of equal volumes of an 8% solution of basic lead acetate and a 16% solution of formalin, and so on). The deparaffinized sections or fixed films are brought up to 70° alcohol and then placed in the dye solution for one hour. The stain solution is prepared by the formula: basic Bismarck brown 0.5 g, ethyl alcohol 80 ml, 1 N hydrochloric acid 20 ml.* The solution is best filtered before use. After removal from the stain, the preparation is passed through three changes of 70° alcohol with one minute in each, and then it is dehydrated, cleared and mounted in balsam. Results: selective, clear staining of the granules of must cells of brown color, with separate granules clearly seen.

The structures of the other cells, including their nuclei, are not stained.

In a preparation stained by the method described it is particularly easy to count the mast cells since the other cells cannot be seen and do not hinder counting.

If it is desired to counterstain the preparation, after the 70° alcohol it is brought down to water and stained by the method of choice.

The method suggested is remarkably simple and does not require the use of differentiation.

SUMMARY

The author describes a method of selective staining of the mast cells granules with the aid of acidified basic Bismark brown solution, prepared according to a special prescription (basis Bismark brown 0.5 gm, ethyl

*Bismarck brown was used by Weigert [1] for staining nuclei, the basic substance of cartilage and mucus. The addition of a certain quantity of hydrochloric acid into our formula is important in principle for, by preventing staining of the nuclei, it provides selective staining of the mast cell granules.

alcohol 80 ml, 1 N hydrochloric acid 20 ml). The method is simple and does not require any differentiation.

LITERATURE CITED

[1] B. Romeis, Microscopic Technique [Russian Translation], Izd. IL, Moscow (1953).

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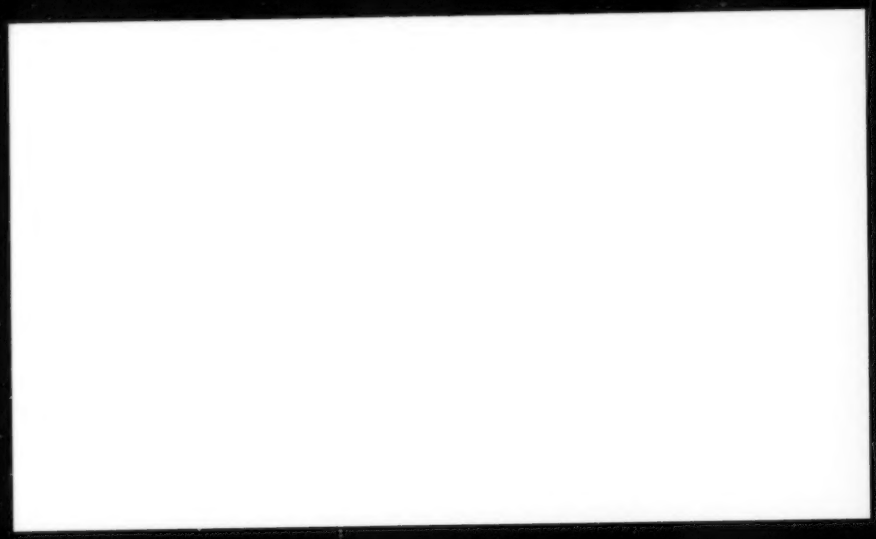

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